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NOVEMBER 1942

VOL. V ISSUED BI-MONTHLY NO. 6

PUBLISHED BY CHARLES C THOMAS

220 EAST MONROE STREET, SPRINGFIELD, ILLINOIS, U.S.A.

JOURNAL OF NEUROPHYSIOLOGY

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Entered as second-class matter February 13, 1939 at the postoffice at Springfield, Illinois, and Menasha, Wisconsin under the Act of March 3, 1879

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ABSENCE OF LOCAL SIGN IN VISCERAL RESPONSES TO PAIN

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(Received for publication July 6, 1942)

A SYMPATHETIC response, manifested by vasoconstriction, piloerection, and sweating, may occur in response to painful, psychic, and other stimuli. In the case of painful stimulation, the question arises whether there is any tendency for localization of the response to the stimulated area. This paper deals with experiments designed to test for localization by measuring responses in man, both near and far removed from the origin of the stimulus.

This physiological information is of interest with reference to questions concerning disturbances of the autonomic nervous system as seen in patients with vasomotor changes and hyperesthesias, resulting from painful scars, causalgias, and other injuries. Livingston (8) reports ten cases of disability caused by pain in the extremities following slight trauma. Vasomotor phenomena, hyperesthesias, referred pain, and in some instances excessive pain were present. Stabins *et al.* (10) have shown that the efferent side of such a reflex need not necessarily be limited to the peripheral distribution in which pain originates.

A series of experiments on vasomotor control and sweat gland activity suggested a logical approach to the solution of the problem concerning localization of the sympathetic response. Many workers have shown conclusively with plethysmographic studies that a decrease in the volume of a part may follow painful, auditory and psychic stimuli (2, 7, 11). An increase in the sweat gland activity, measured in terms of skin resistance change, has been demonstrated with different types of stimulation (2, 3, 4).

By using methods for studying finger volume changes and skin resistance changes in the right and left hands of an individual, and by applying the painful stimulus to the dorsum of either hand, any tendency for localization of the response should be apparent. If any tendency for localization were present, the finger tip of the left hand should decrease in volume to a more pronounced extent than the corresponding finger tip of the right hand, when the left hand was stimulated and *vice versa*. Skin resistance changes were used as an additional check.

METHODS

Changes of finger volume were recorded optically, using a modification of the method described by Burton (2). The left and right index fingers (second and third phalanges) were placed in separate snugly fitting brass tubes, the junction between finger and plethysmograph being sealed with liquid adhesive compound. The plethysmographs were in turn connected by rubber tubing to a tambour, fitted with a rubber diaphragm and a small mirror chip. By air displacement, changes in finger volume caused a deflection of a light

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beam, which was reflected from a slit lamp to a recorder carrying sensitized paper. The tambour carrying the mirror chip was placed at a distance of one meter from the recorder. The two systems were equilibrated so that an equal change in each finger would cause an equal deflection of the light beams on the recorder aperture. Calibration of the plethysmographic response was done by causing a known volume of mercury, in a manometer, to displace an equal volume of air in the plethysmograph system, recording the resulting deflection of the light beam on the recorder. By this method the responses obtained could be expressed in volume change per cc. of finger mass. The latter was measured by water displacement. The possibility of leaks in the air conducting system was checked before each recording. Diminution of finger volume has been shown to occur on deep breathing (1). Assuming that the initiation for this reflex is common to both right and left index fingers, one might expect an identical response from both sides, as a result of a deep breath. If both systems were of the same sensitivity and free of leaks, a deep breath caused identical excursions of the light beams. Figure 1 is a typical curve illustrating such a response. This was found to be valid by checking with the volumetric method described above. Since the response resulting from a deep breath may be great at times, respirations were recorded optically during each experiment to obviate confusion with responses obtained following painful stimuli.

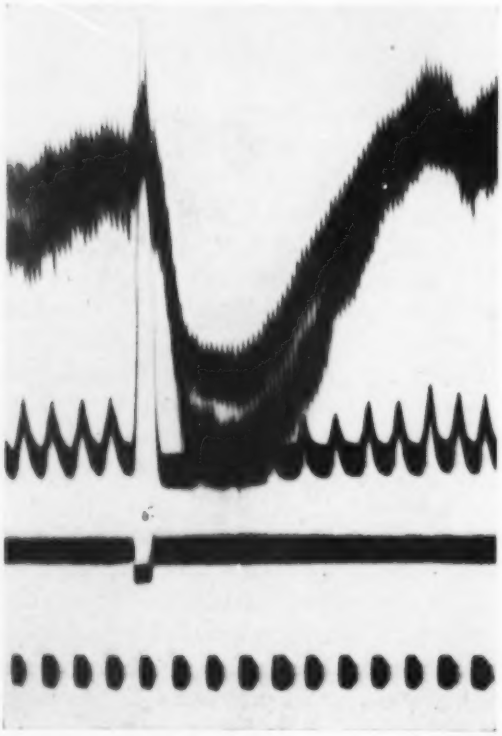

FIG. 1. This record shows the equal vasoconstriction obtained in both right and left index fingers following a deep breath. The uppermost curve is the right index finger, the second curve the left index finger. Respiratory excursions are recorded on the third line and the signal marker on the fourth line. The bottom line is time recorded in 5 sec. intervals.

Skin resistance changes were recorded* by the method of Geohegan *et al.* (5) on a two channel instrument (in place of the oscillograph), each channel consisting of a Wheatstone bridge, a direct-current amplifier and a magnetically operated stylus.† Tracings were made on stylograph paper (paper covered with a thin coating of wax) by the warm point of the stylus. Leads from the right and left ring fingers were connected to the proper channel of the instrument, the ground electrode being held under the tongue of the subject. Small zinc electrodes were applied over a thin coating of Cambridge electrode jelly. A uniform contact was maintained by fixation with scotch tape.

Painful stimuli were given according to a method of Hardy and Wolff (6). The light of a 1000-watt bulb was focused by two plano-convex lenses, and allowed to irradiate an area 3^2 cm. on the dorsum of either hand for 10 sec. By such a control of intensity and duration, a uniform painful stimulus was delivered in all of the experiments. Stimulations were done on both right and left hands during each experiment.

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* A word of thanks is given to Dr. Orlando J. Aidar whose assistance in this portion of the experiments made the technical procedures more easily executed.

† This machine was designed in this laboratory by W. A. Geohegan and built by the Rahm Instrument Company of New York.

Twenty-five experiments were carried out on twenty healthy first year medical students (male and female). Observations were usually done in the morning following breakfast. Each subject was blindfolded before the experiment began so that stimulations would not be anticipated. After the recording equipment had been applied, the subject was given a 15 minute rest period before starting the experiment. All experiments were done in a controlled temperature room at 26° C.

Results

In all the subjects tested there was no evidence suggesting a difference in the amplitude of the sympathetic responses in the right and left hand. Recordings of skin resistance changes were made in 12 subjects simultaneously with finger volume recordings, and positive responses were obtained in all of these. Marked decreases in finger volume were obtained in 18 of the 20 subjects. The remaining 2 subjects had similar responses but these were consistently smaller. The individual variations in the amplitude of the response was thought to be due to a difference in the individual as far as stability of the autonomic nervous system was concerned, and also to be dependent on the physiological status of the subject at the time of the recording. Experiments were repeated on a few of the subjects at a later date. Some subjects yielded greater responses to stimulation, while others had smaller responses, when compared to the previous studies.

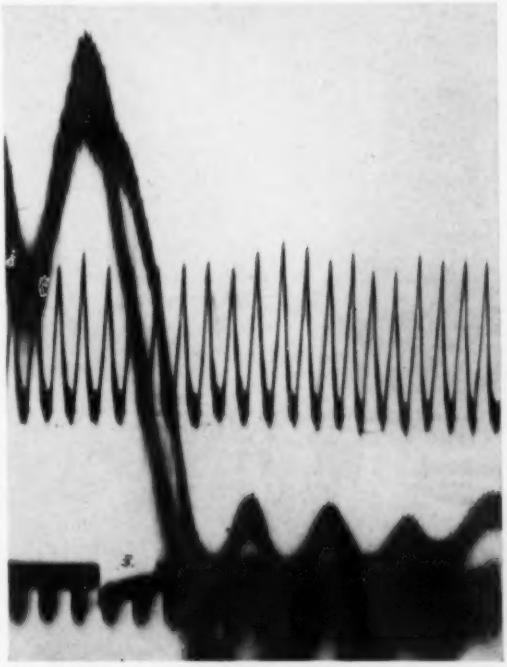

Fig. 2. Top curves are finger volume recordings of left and right index fingers with the right finger curve uppermost. Respiratory excursions are on the second line, with signal marker and time in 5 sec. intervals on the bottom line. The decrease in finger volume represented is approximately 0.01 cc. Note that the left and right fingers constricted equally to a painful stimulus of 10 sec. applied to the dorsum of the left hand.

Figure 2 is a typical curve of left and right index finger volume change following painful stimulation applied to the dorsum of the left hand. It is apparent that the volume decrease in the left index finger is identical with that of the right. This relationship held true in all responses obtained with an equal number of stimulations being done on the left and right hand of each subject. Stimulation of different sensory areas on the dorsum of the hand caused no differences in the responses. In Fig. 3 the light beams were

purposely superimposed to facilitate detection of any disparity in the responses of left and right. It is evident that the recording beams followed an identical path throughout.

In twelve of the subjects, while simultaneous recordings were being made of volume changes, skin resistance changes were also being measured. Figure 4 is a typical record of the changes of skin resistance following painful stimulation. The curves follow the same contour throughout. By calibrating the instrument against a known amount of resistance change, it was possible

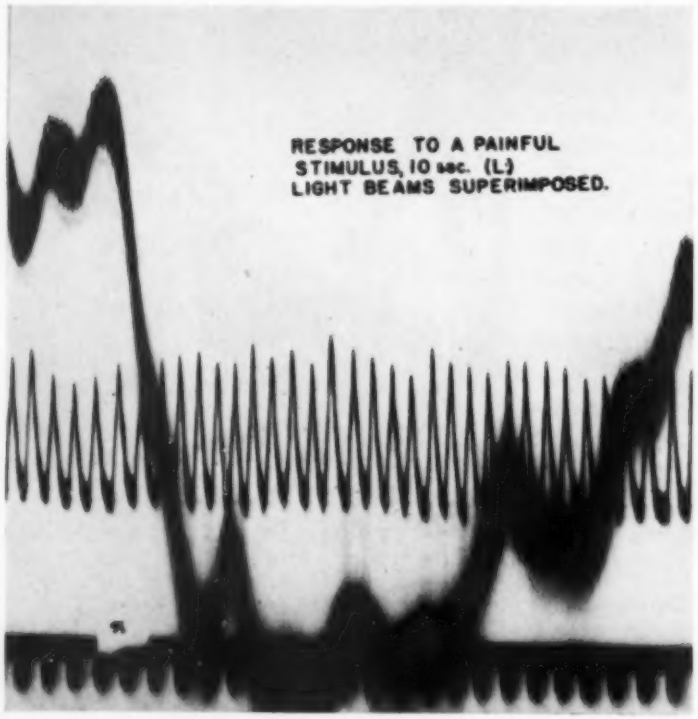

FIG. 3. Vasoconstriction in the right and left index fingers following a 10 sec. painful stimulus on the dorsum of the left hand. The light beams were superimposed in this record. It is evident that the two curves are identical since they appear as one. Respiration is recorded on the third line, signal marker on the fourth line with the time recorded in 5 sec. intervals on the bottom line.

to express the response as percentage change of the original resistance. Although the contours of the curves were similar, variations in the actual percentage change was found.

In three of the subjects, a greater response occurred always on the right side; the reverse was true in 3 other subjects. Of the remaining 6, the greater response occurred as often on the left as on the right, and in many instances the two responses were equal. Differences in the percentage changes between left and right were usually from 2–5 per cent. Differences as great as 10 per cent were observed on several stimulations. It should be emphasized here again that an equal number of stimulations was carried out on the right and left hands of each individual. The fact that the responses follow the same contour, and that the greater response (when obtained) could not be consistently demonstrated on the stimulated side, indicates little tendency for localization of this type of response.

Discussion

The outstanding factor concerning the autonomic nervous system is its tendency to go into action as a whole in the face of immediate danger or in severe environmental change. Such responses occur in severe physiological stresses of the individual. Other situations have been pointed out, in which one might expect "local sign" to appear. Although these experiments show such phenomena to be lacking for the particular groups studied, they do

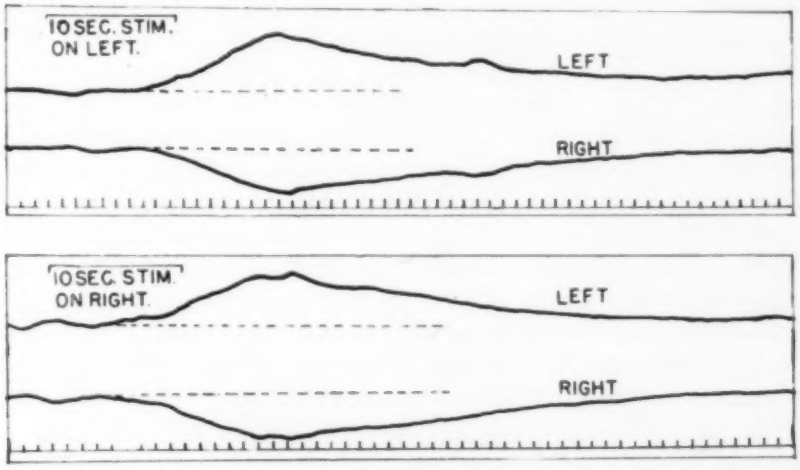

Fig. 4. Tracings of recordings of skin resistance changes in left and right ring fingers in response to a painful stimulus of 10 sec. applied first to the dorsum of the left hand and then to the dorsum of the right hand. The time is in one second intervals. Dotted lines represent projected basal resistances. The instrument was so arranged that a decrease in resistance in both channels would cause the recording styluses to diverge. It will be seen that the curves follow the same contour. The responses shown represent a 21 per cent change from a basal resistance of 19,000 Ω.

not necessarily obviate the occurrence of such in other instances. A prolonged stimulation, much less intense than was given here, might serve to initiate localization in a part. Other types of stimulation such as deep pain, repeated at frequent intervals, could result in prolonged vasoconstriction in a single part. In this series of experiments, a psychic stimulus (produced by telling the subject that he would receive an intolerable burn) caused as much vasoconstriction and skin resistance decrease, and in some instances more, than when an actual painful stimulus was applied. Neumann (9), in observing unmolested subjects over long periods of time with plethysmographic studies, has noted that although the spontaneous volume changes which occur in identical parts may follow identical paths for long periods, they may for a time drift apart. Occasionally the volume changes may be in the opposite direction in these subjects. Our observations have been directed to the response obtained after strong painful stimuli; spontaneous curves seen in these responses have followed one another closely.

A question has been raised concerning the decrease in skin resistance obtained following stimulation. Some believe that this response is purely cholinergic (4), *i.e.*, evidence of increased sweat gland activity alone. Others think that the response is due to blood vessel changes (adrenergic response), plus increased sweat gland activity. Carmichael (3) states that the skin resistance change can be elicited in the presence of one or the other, but is abolished in the absence of both vascular activity and increased sweat gland activity. Regardless of the interpretation placed upon skin resistance changes, it is an interesting fact that there was no significant difference in the responses obtained in the 2 sides of the body.

Summary

A series of experiments, designed to demonstrate localization of a sympathetic response in man following painful stimulation has been presented. Left and right index finger volume changes, obtained simultaneously, failed to show any difference in amplitude following a unilateral painful stimulus. Skin resistance changes recorded at the same time from the ring fingers bear a similar relationship. Although variations in percentage change of original skin resistance were observed at times, the greater change was not consistently present in the stimulated hand. These experiments indicate that there is no tendency for localization of the responses studied.

I wish to express my sincere thanks to Dr. J. C. Hinsey for his suggestions and assistance during the course of these experiments.

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POTENTIALS RECORDED FROM THE NERVE TRUNK AND THE DORSAL ROOT BY MICRO-ELECTRODES

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(Received for publication July 9, 1942)

MICRO-ELECTRODES were used by Renshaw, Forbes and Morison (4) to record the potential changes that occurred in various regions of the brains of chickens, rabbits and cats under different experimental conditions. Similar electrodes were used by Therman, Forbes and Galambos (5) in a study of the response of the superior cervical ganglion of the cat to preganglionic stimulation. The results in the two groups of experiments suggested that micro-electrodes might provide the means by which definite information could be obtained concerning the electrical potential developed at the cell body of the neuron.

The anatomical arrangement obtaining in spinal ganglia, where the majority of the cell bodies are situated in a layer surrounding the fiber trunk, appeared to render these structures particularly suitable for a study of the potential developed by single cell bodies. The absence of typical synapses in these ganglia should also eliminate the complications of post-synaptic activity. Accordingly studies were begun on these ganglia using the glass micro-electrode described by Therman, Forbes and Galambos. The potential records were so complex and the interpretation so uncertain that a study of the potentials developed by the nerve trunk, as recorded by micro-electrodes, was made in an attempt to establish a basis for the interpretation of the records from ganglia.

METHODS

In all instances cats anesthetized with dial urethane (Ciba) were the experimental animals employed. The dorsal roots with their ganglia were exposed by laminectomy. The roots of the first and second sacral and the sixth and seventh lumbar segments are long and the ganglia readily accessible and therefore suitable for study of both ganglionic and fiber activity. These roots were used in all experiments. For recording the potentials of nerve trunks either the whole sciatic nerve or one of its bundles or a dorsal root, central to the ganglion, was the site for the electrode. In a few instances the sciatic nerve was excised, placed in a bath of Ringer's solution, and the potentials developed on stimulation recorded for comparison with those obtained with the nerve *in situ*.

Several arrangements of electrodes were employed for recording the action potential. A pair of silver-chlorided silver hooks spaced 1 cm. apart were used either with intact nerve on both electrodes or with the nerve killed at the distal electrode. When these electrodes were used, the stretch of nerve on them was freed from the rest of the tissue as much as possible. Also a single silver hook under the nerve, or a silver wire or disc on its free surface, was used in combination with a diffuse distant electrode consisting of a silver plate buried between skin and muscle on the side opposite to the location of the discrete electrode. Micro-electrodes were arranged to record against a distant diffuse lead or against a silver hook on the killed end of the nerve or another micro-electrode.

The micro-electrodes were of the glass type filled with Ringer's solution. They were placed as desired in the nerve or ganglion by means of micro-manipulators. The recording

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* Porter Fellow of the American Physiological Society.

system was the cathode-ray oscillograph and associated amplifiers described by Therman, Forbes and Galambos.

Controlled activity in the nerve or ganglion was obtained by stimulation of the sciatic nerve or one of its branches peripheral to the site of the recording electrodes. The stimuli were condenser discharges controlled by a neon tube circuit and passed through a special transformer. These stimuli were applied once per second and were synchronized with the cathode-ray sweep by having the stimulator tripped by the cathode-ray sweep circuit.

Results

Nerve. The potentials developed by a stimulated nerve trunk when it is isolated and placed on the conventional paired electrodes have been

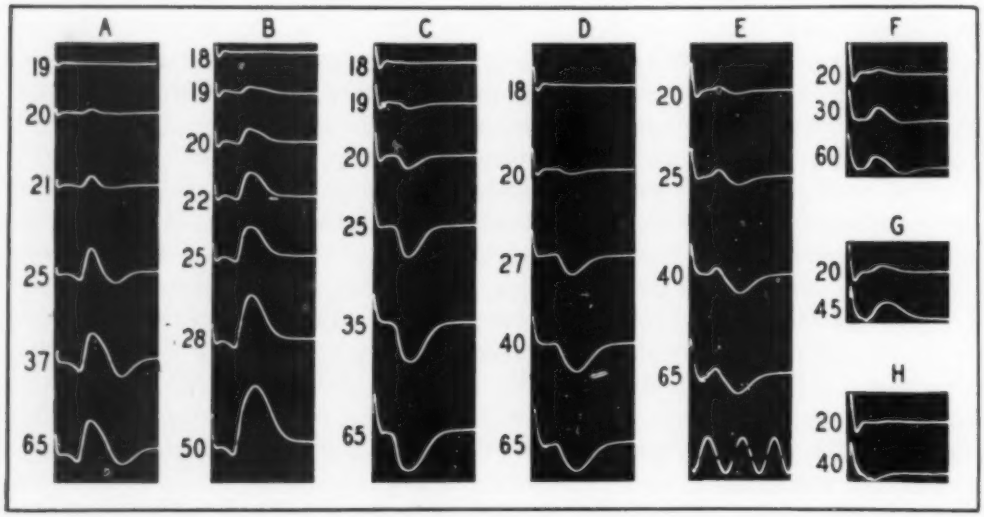

Fig. 1. Comparisons of the potentials recorded from a stimulated nerve trunk by a pair of large electrodes and by a 30μ micro-electrode.

The numeral to the left of each record indicates the strength of the stimulus. A, intact nerve on a pair of large electrodes spaced 1 cm. apart. B, nerve over distal electrode of the pair killed. C, 30μ micro-electrode substituted for proximal electrode of pair, 1 cm. from the border of the killed part of nerve. D, 30μ electrode 6 mm. from border of killed nerve. E, 30μ electrode 4 mm. from border of killed nerve. F, 30μ electrode 2 mm. from the border of killed nerve. G, 30μ electrode at junction of intact and killed nerve. H, 30μ electrode well within killed nerve. Time record in E is 2000 cycles/sec. and applies to all curves.

thoroughly studied, and the form of the potential record obtained under these conditions is well known. The potential changes in the stimulated nerve as recorded (i) by paired large electrodes and (ii) by micro-electrodes are shown in Fig. 1. When the intact nerve is placed on a pair of electrodes, spaced 1 cm. apart, the anticipated diphasic potential is recorded (Fig. 1-A). When the part of the nerve overlying the distal electrode has been killed the potential change is monophasic (Fig. 1-B). The records of Fig. 1-A and B are identical with those from completely isolated nerve except that there is a small initial positive deflection.

Figure 1-C shows the records obtained when a 30μ electrode is substituted for the proximal lead of the pair used in the previous experiments. The 30μ electrode was so connected that activity (negativity) in the nerve under it gave a downward deflection. The micro-electrode was first placed 1 cm. from the border of the killed stretch of nerve which rested on a large silver electrode. The records are identical in form with those obtained with paired electrodes but are somewhat reduced in amplitude.

Figure 1, D to H, demonstrates the change in form which the potential curve undergoes as the micro-electrode is brought nearer the killed part of the nerve. When the micro-electrode is a suitable distance away from the killed part of the nerve a typical monophasic record is obtained (Fig. 1, C and D). As the distance between the electrode and the killed nerve is decreased an initial positive wave is recorded. This is first seen at 4 mm. distance (Fig. 1-E) and increases as the negative phase decreases until it is the only deflection recorded (Fig. 1, F and G). When the micro-electrode is well within the killed stretch of nerve only the shock artefact is recorded (Fig. 1-H). The presence of a positive wave alone indicates that the nerve impulse dies out just before reaching the position of the electrode. The same change in the form of the potential record occurs when gross electrodes are similarly arranged on the nerve.

The potential curve recorded by the 30μ electrode (Fig. 1-C) grows as the strength of the stimulus is increased in the same way as that recorded by gross electrodes (Fig. 1-B). There is no sudden appearance of a spike which persists over a considerable range of intensities of stimulation as would be expected if the micro-electrode were recording from a single fiber or a very small group of fibers. All the characteristics of the curves are such as to suggest that the micro-electrode was recording the summed potentials from all of the fibers of the nerve trunk.

All micro-electrodes with tip diameters of 15μ or larger gave potential records similar to those recorded by gross electrodes. A 2 mm. silver disc on the nerve trunk, when the other electrode is distant and diffuse, records the potential changes as in Fig. 4, III-c and d. The triphasic curve is identical with that obtained with a 66μ electrode recording against a distant diffuse one (Fig. 4, III-g and h) or a 16μ electrode similarly arranged. This form of curve is typical of the combination of a discrete electrode on the active tissue and a distant diffuse one when the discrete electrode is on the nerve some distance from both the site of origin and of termination of the nerve impulse (1, 2).

Selectivity of micro-electrodes. To be of greatest service a micro-electrode should be sufficiently selective to record only the potential set up in the element immediately under its tip. The experiments of Renshaw, Forbes and Morison suggested that this was realized in certain parts of the central nervous system. The conditions in the nerve trunk when many fibers are activated synchronously are quite different from those in the central nervous system where activity may be very asynchronous. It is, therefore, necessary

to establish by experiment the extent of selectivity attained by micro-electrodes when placed in a nerve trunk where many fibers are activated synchronously.

An approximate estimate of the actual selectivity (attenuation of potentials arising at a distance) of micro-electrodes was obtained by placing the electrode in or near a small nerve bundle of the sciatic trunk and recording the potential change between it and a distant diffuse electrode. Data showing the attenuation of the voltage as the electrodes were moved away from the active bundle are summarized in Table 1. When an excised nerve placed

Table 1. Voltage recorded by micro-electrodes of different tip diameters at various distances from the active nerve fiber.

Distance of active electrode from bundle	Electrode diameter in μ	Relative voltage	Stimulus strength, dial reading
0.0 mm.	42	100	15
3.0	42	40	15
5.0	42	36	15
0.0	42	100	50
0.2*	42	63	50
3.0	42	47	50
6.0	42	42	50
0.0	16	100	20
1.0	16	10.7	20

* The electrode was in the connective tissue surrounding the bundle and may have been between 0.1 mm. and 0.3 mm. from the active fibers.

in a bath of Ringer's solution was employed the attenuation of the potential was much greater with a micro-electrode of a given size than for the same electrode with the nerve *in situ*.

An appreciable part of the potential produced by the active bundle can obviously be recorded with the electrode some distance away. The larger the electrode tip the greater the potential recorded at a given distance. Even with the smaller electrode (diameter 16μ) the potential when the electrode was 1 mm. away was $\frac{1}{10}$ that recorded when the electrode was in the active bundle. This is far less attenuation than is required for discriminating single fiber activity. It is not surprising that we failed to record single fiber responses from a nerve trunk, in which many fibers are active, with micro-electrodes whose tip diameters are 15μ or larger. However, the marked attenuation of the voltage recorded by a 16μ electrode as compared to that recorded by a 42μ electrode suggests that smaller electrodes may be desirable.

The use of two micro-electrodes. Records obtained when two micro-electrodes were used are shown in Fig. 2. The use of two micro-electrodes

did not increase the selectivity. Compare the curves recorded between a micro-electrode and a diffuse one (Fig. 2-1, 2, 3) with those recorded between two micro-electrodes (Fig. 2-4, 5, 6, 8, 9, and 10). The two micro-electrodes attenuated the voltage and slightly increased the complexity of the curves. Stronger stimuli increased these complexities to the point where the records could not be interpreted (records not shown). The complexities arise from

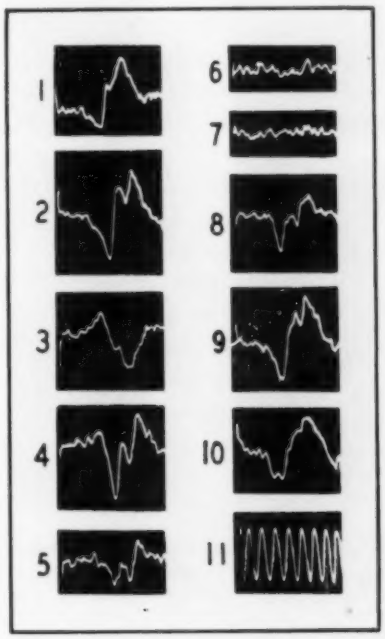

FIG. 2. Action potentials from the sciatic nerve as recorded by two micro-electrodes.

1, 2 and 3 between each of the micro-electrodes and a diffuse one; 4) recorded between a 42μ and a 32μ electrode, separated 1 cm. longitudinal to nerve trunk; 5) same electrodes as 4 separated 5 mm.; 6) same electrodes as 4 separated 2 mm.; 7) noise level, no stimulus; 8) electrodes separated 2 mm., stimulus increased to 3 times the value in 6; 9) electrodes perpendicular to longitudinal axis of nerve, separated 2 mm.; 10) electrodes separated 5 mm.; 11) time record 5000 cycles/sec.

the activity of different fibers under the two electrodes. Differences in conduction rate cause slight differences in the time of arrival of the nerve impulses at the two electrodes and sharp spikes appear in the record. These variables render the use of two micro-electrodes impractical in the nerve trunk even though the potentials from distant sources are cancelled.

The dorsal root ganglia. As a site for the study of cell body potentials the dorsal root ganglia seemed favorable. However, the variable contribution from nerve fibers rendered the potential records complex. The foregoing studies on the nerve trunk were made in an attempt to evaluate the fiber contribution. The conclusion was reached that electrodes greater than 15μ in diameter do not record single fiber responses. This conclusion usually applies to the ganglia also.

One type of record obtained from the ganglion when a micro-electrode is used with a diffuse one is shown in Fig. 3. When the electrode is on or just within the surface layer a triphasic wave is recorded (Fig. 3, A and B). As the electrode penetrates the ganglion more deeply the negative spike is re-

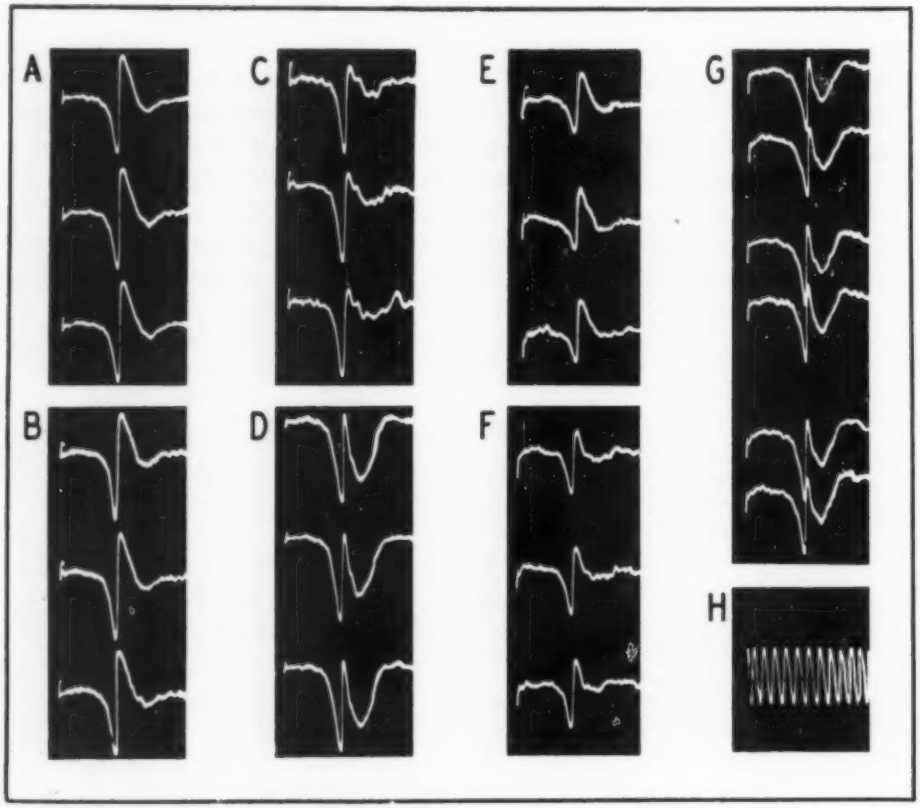

FIG. 3. Potentials from dorsal root ganglion recorded by a micro-electrode.
A. Potential recorded between a 20μ electrode and a distant diffuse one. Micro-electrode on surface of ganglion. Stimulus 20.
B. 20μ electrode just penetrating connective tissue covering of ganglion.
C. 20μ electrode about 0.7 mm. in ganglion.
D. Electrode deep in ganglion, ca. 1.5 mm.
E. Distant electrode replaced by a second micro-electrode on the connective tissue covering of ganglion. Electrodes separated about 1 mm. Original micro-electrode just penetrating ganglion.
F and G. Two different positions of original micro-electrode deeper in the ganglion than in E.
H. Time record, 4000 cycles/sec.

duced and a prominent later positive potential is recorded (Fig. 3, C and D). When the diffuse electrode is replaced by a second micro-electrode situated on the connective tissue layer outside the ganglion, records of similar form are obtained (Fig. 3, E, F, and G).

A reasonable interpretation of these records is that the "initial phases" (consisting of the initial positive deflection and the succeeding negative deflection as far as its crest) represent activity in the nerve fibers. The duration of the negative wave is slightly increased when the micro-electrode is on or just within the surface layer of the ganglion. The descending portion of the negative wave then represents summed potentials contributed in part

by nerve fibers and in part by cell bodies. When the electrode penetrates the ganglion more deeply its tip passes beyond many of the cell bodies and lies among the axon branches to these cell bodies. The late positive wave recorded from the depths of the ganglion probably represents the difference of potential existing between the cell bodies and their axonal connections. The part of the cell body attached to the axon branch must be activated first by the nerve impulses. The radial arrangement of the cell bodies makes it possible that part of the positive wave represents the difference of potential existing between two parts of the cell body as the impulse spreads over it. When the electrode is centrally located inside the spherical layer of cell bodies (near the collected axon branches) the positive wave should theoretically be greatest. This was the probable location of the electrode tip when large positive potentials were found experimentally.

The interpretation that the "initial phases" of the curves are produced by nerve fibers is supported by the reasonable value of the conduction rate (ca. 90 m/sec.) calculated on this assumption. The duration of the late positive phase (1 msec.) supports the interpretation that it is contributed by cell bodies. This value is comparable to that found by Renshaw (3) for the duration of activity in motoneurons of the spinal cord. The smooth contour of the curves suggests that the records represent the summed potentials from many units. The late positive wave from the ganglion is homologous with the terminal positive phase from the nerve trunk, but greater in amplitude and duration.

The interpretations regarding the sources of the recorded potentials are further supported by the observation that with a 2 mm. disc on the surface of the ganglion there is recorded a small later negative wave (Fig. 4, I-a). The same late negative wave is present when a 25μ electrode replaces the disc (Fig. 4, I-b). A large positive wave, out of which negative spikes rise, follows the "initial phase" when the micro-electrode is pushed into the surface layer of the ganglion (Fig. 4, I-c). When the stimulus is increased in strength the late negative spikes increase in size (Fig. 4, I-d). Our interpretation is that the positive wave represents the difference of potential existing between many cell bodies and their axon branches, and the negative spikes represent the development of negativity in a few cell bodies near which the tip of the electrode rests. The increase in size of the negative spike with a stronger stimulus indicates that more nearby cell bodies are activated.

When the 25μ electrode penetrates more deeply into the ganglion the positive wave persists but the negative spikes disappear (Fig. 4, I-e and f). Now the electrode has passed beyond the layer of cell bodies and its tip is surrounded only by axon branches. The positive wave means that these axon branches and proximal ends of the cell bodies are recovering as the cell body is invaded by the wave of depolarization.

Replacement of the diffuse electrode by a 25μ electrode in the same location attenuates the potential record. Strong stimuli elicit small negative spikes during the positive phase (Fig. 4, II-a and b). When the distant 25μ

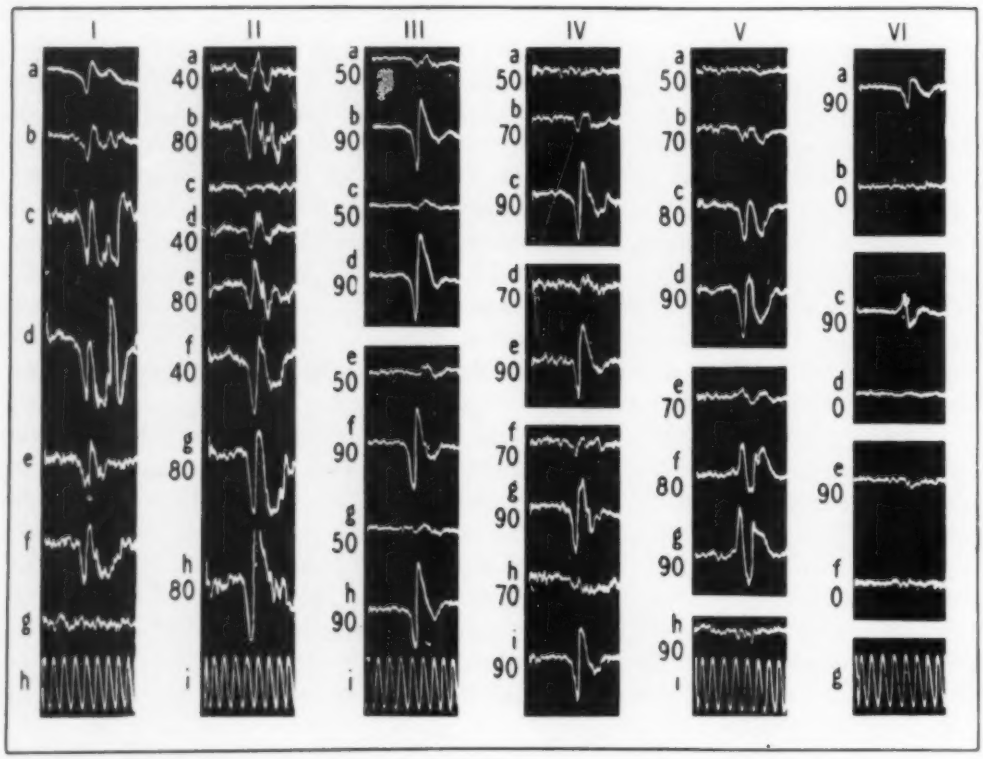

FIG. 4. Potentials from ganglia and dorsal roots.

I. a) 2 mm. silver disc on ganglion, other lead diffuse; negativity at disc deflection upward. Stimulus 40.
b) 25μ electrode on surface of ganglion, other conditions as in (a).
c) 25μ electrode penetrating upper surface of the ganglion, other conditions as in (a).
d) Stimulus increased to 80, otherwise as (c).
e) Micro-electrode pushed deeper in the ganglion, stimulus 40.
f) Stimulus increased to 80, otherwise as (e).
g) Noise level, no stimulus.
h) Time record, 2000 cycles/sec.

II. a) 25μ electrodes replace diffuse lead, 25μ electrode in ganglion same position as in (I-g).
b) Stimulus increased to 80, otherwise as (a).
c) Noise level, no stimulus.
d) Two 25μ electrodes, peripherally placed one penetrating ganglion; centrally placed one on surface of ganglion; negativity at peripiheral electrode deflection up. Stimulus 40.
e) Stimulus 80, otherwise as (d).
f) Centrally placed electrode now moved to a position medial to other micro-electrode. Penetrating electrode, lateral, and a diffuse one. Stimulus 40.
g) Stimulus 80, otherwise as (f).
h) Record between the two micro-electrodes. Stimulus 80.
i) Time record, 2000 cycles/sec.

III. a) 2 mm. silver disc on surface of ganglion against diffuse electrode. Negativity at disc upward deflection. Stimulus 50.
b) Stimulus 90, otherwise as (a).
c) Silver disc placed on dorsal root central to ganglion. Stimulus 50.

electrode is moved to a position on the ganglionic surface 1 mm. central to the penetrating electrode the recorded curve is of the same general form (Fig. 4, II-d and e). Accidental displacement of the penetrating electrode has probably brought the tip nearer a few cell bodies so that now small negative spikes appear in the later part of the record. With the penetrating 25μ electrode in the same position and a diffuse lead, the positive wave is larger and shows traces of the negative spikes (Fig. 4, II-f and g). The record between the deep micro-electrode and another on the ganglionic surface medial to it shows a large negative wave followed by a small positive wave out of which small negative spikes rise. The form of all the potential curves presented in this group can readily be explained on the basis of the interpretation regarding the origin of the potentials set forth.

This interpretation also explains the form of the curve recorded from the ganglion by a 2 mm. disc (Fig. 4, III-a and b), and from the dorsal root (Fig. 4, III-c and d). The same explanation applies to the records obtained with a 66μ electrode on the ganglion (Fig. 4, III-e and f) and on the root

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d) Stimulus 90, otherwise as (c).
e) 66μ electrode on surface of ganglion against diffuse lead. Stimulus 50.
f) Stimulus 90, otherwise as (e).
g) 66μ electrode placed on dorsal root central to ganglion. Stimulus 50.
h) Stimulus 90, otherwise as (g).
i) Time record, 2000 cycles/sec.

IV. a) 20μ electrode on surface of ganglion. Stimulus 50.
b) Stimulus 70, otherwise as (a).
c) Stimulus 90, otherwise as (a).
d) 30μ electrode placed on dorsal root. Stimulus 70.
e) Stimulus 90, otherwise as (d).
f) 20μ electrode on surface of ganglion. Stimulus 70.
g) Stimulus 90, otherwise as (f).
h) 20μ electrode placed on dorsal root. Stimulus 70.
i) Stimulus 90, otherwise as (h).
Time record from III-i.

V. Two micro-electrodes penetrating ganglion, separated 1 mm. longitudinally. Negativity at 30μ electrode deflection up, at 20μ electrode deflection down.
a) Record between 30μ electrode and diffuse one, stimulus 50.
b) Stimulus 70, otherwise as (a).
c) Stimulus 80, otherwise as (a).
d) Stimulus 90, otherwise as (a).
e) Record between 20μ electrode and diffuse one, stimulus 70.
f) Stimulus 80, otherwise as (e).
g) Stimulus 90, otherwise as (e).
h) Record between 20 and 30μ electrodes, stimulus 90.
i) Time record, 2000 cycles/sec.

VI. 20μ and 30μ electrodes in dorsal root.
a) 30μ electrode and diffuse one, stimulus 90.
b) Noise level, no stimulus.
c) 20μ electrode and diffuse one, stimulus 90.
d) Noise level, no stimulus.
e) Record between 20μ and 30μ electrodes, stimulus 90.
f) Noise level, no stimulus.
g) Time record, 2000 cycles/sec.

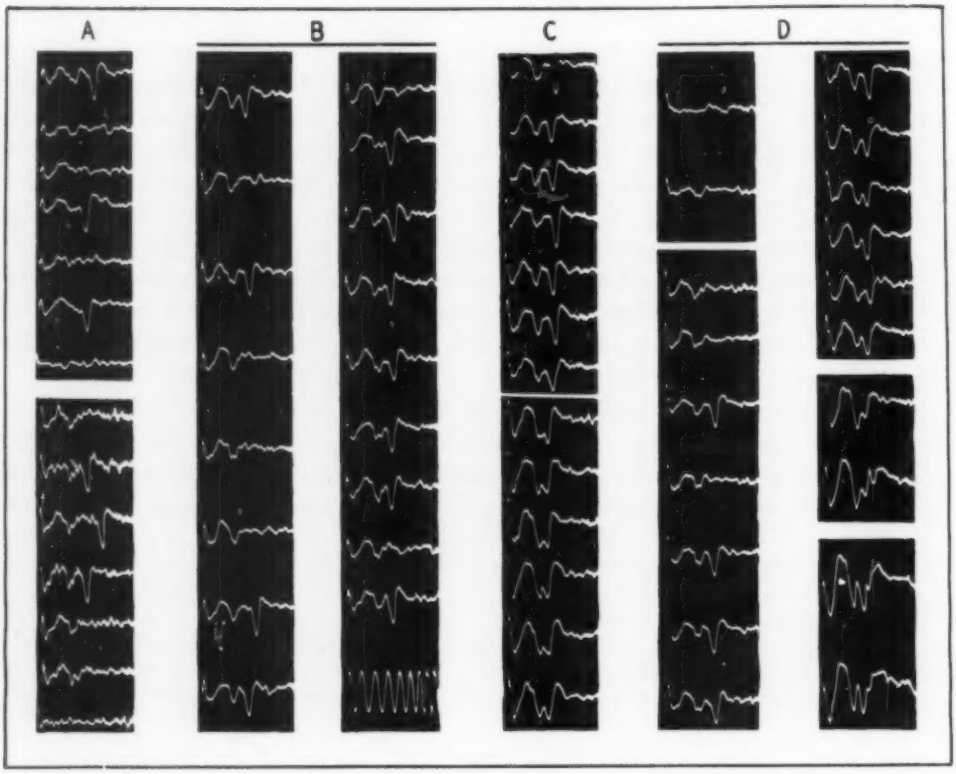

FIG. 5. Potentials recorded from single units of the dorsal root ganglion by micro-electrodes.

A. Two 20μ electrodes. One electrode just penetrating the surface layer of the ganglion. The second outside connective tissue covering of the ganglion. Negativity of penetrating electrode gives downward deflection. Upper portion, stimulus 12.5. Lower portion, as upper, but high frequency filter not used.

B. Stimulus 14; filter used throughout, otherwise as A.

C. Upper portion, stimulus 18. Lower portion, stimulus 20.

D. Selected portions of record obtained when the stimulus was increased from below threshold to maximal. Portions selected show characteristic changes in the potential record. Electrodes as in A. Time record 2000 cycles/sec.

(Fig. 4, III-g and h). With a 30μ electrode on the ganglion (Fig. 4, IV-a, b and c) and on the root (Fig. 4, IV-d and e) and also with a 20μ electrode similarly placed (Fig. 4, IV-f to i) the same form of curve is recorded with a slight attenuation of the potentials. The small late negative wave of the records is contributed by the cell bodies. It is small and smooth because the electrode is relatively far away from individual cell bodies not all of which become active at the same time.

Two micro-electrodes separated 1 mm. in an antero-posterior direction were pushed deeply into the ganglion. The curves recorded between the 30μ electrode and a diffuse one are shown in Fig. 4, V-a to d; those recorded between a 20μ electrode and a diffuse one in Fig. 4, V-e to f, and the curve

recorded between the two micro-electrodes in Fig. 4, V-h. A late positive wave characteristic of deep penetration is recorded by both electrodes. Between the two electrodes essentially no potential is recorded, which indicates that the potentials arose from sources equi-distant from both electrodes and were cancelled between them. The same pair of electrodes when placed on the dorsal root recorded potentials as seen in Fig. 4, VI-a to f. The records from both ganglion and root are of the form expected on the basis of our interpretation regarding potential origin.

In a few cases potentials that we interpret as the response of single units were obtained (Fig. 5). The negative spike (deflection downward) appeared with threshold stimuli (Fig. 5-A) and was still present with the same amplitude with maximal stimulation (Fig. 5-D, later portion of the record). The spike voltage is about 50μV. When the stimuli were near threshold the latency varied between 2 and 2.6 msec. (Fig. 5, A). This latency variation is characteristic of the single unit response when subjected to threshold stimulation. With stronger stimuli (Fig. 5, C, lower portion) the latency became constant at 1.7 msec. These stimuli also elicited another earlier spike of about the same voltage, and having a latency of about 1.4 msec. Since the spikes are negative they represent activity in cell bodies at the electrode tip. The presence of the two spikes indicates the location of the electrode tip near two closely approximated cells. That the selectivity of a 20μ electrode is adequate to record from one or two individual cell bodies is probable because the diameter of these cell bodies may be as much as 100μ. Therefore, when a 20μ electrode rested on two of them lying side by side it would be well isolated from most of the others.

On the basis of the conduction rate usually observed in these nerve fibers the nerve impulse should have reached the electrode in 1.1 msec. At constant latency of response the additional delays observed were for the early spike 0.15 msec. and for the later spike 0.4 msec. The early spike began 1.25 msec. and the later one 1.5 msec. following the shock artefact. The observed delay may be explained by a uniform but slow rate of conduction in the two fibers with a marked difference in rate for the two. The calculated rates are for the early spike 80 m/sec. and for the late one 66 m/sec. However, with stimuli as weak as those just necessary for constant latency of response in these units, we have never observed a conduction rate as slow as 80 m/sec. from the nerve trunk. A more probable explanation is that the rate of conduction was nearly the same in the two fibers as well as in the cell bodies, but that the rate of propagation is slower in the cell body than in the nerve fiber. The difference in the delay might then be due to differences in the distance from the point of entry of the impulse into the cell body to the point of contact with the electrode.

According to the interpretation of the origin of the ganglionic potentials that we have made, the spike represents negativity at the point of contact between cell body and electrode as the wave of depolarization invades the cell body. The same invasion of the cell bodies by the wave of depolarization

gives rise to the positive wave when the electrode is deeper in the ganglion, near the axon branches (Fig. 3).

Summary

Micro-electrodes have been used to record potentials from nerve trunks and from dorsal root ganglia.

1. The form of the potentials recorded from the nerve trunk by micro-electrodes is the same as that recorded by gross electrodes similarly arranged (Fig. 1).

2. The records are easier to interpret when one micro-electrode is used in combination with a distant diffuse one.

3. Micro-electrodes whose tip-diameters are greater than 15μ are not sufficiently selective to record single fiber responses from the nerve trunk when large numbers of the fibers are activated synchronously.

4. Small electrodes (16μ) attenuate potentials arising at a distance far more than do large electrodes (42μ).

5. The ganglionic potential curve is very complex when recorded by large micro-electrodes within the ganglion (Fig. 3 and 4). It is composed of waves representing activity of the nerve fibers contained in the ganglion and others representing activity of the cell bodies.

6. The various phases of the complex wave have been assigned to the various structures, and the alterations in the form of the curve with depth of penetration of the electrode explained.

7. In a few instances single cell-body potentials have been recorded from the ganglia (Fig. 5). This cell-body spike has a greater latency than can be explained by the conduction time in the nerve fiber and this delay probably represents a slowing of the depolarization wave as it invades the cell body.

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MEDIATION OF DESCENDING LONG SPINAL REFLEX ACTIVITY*

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(Received for publication July 9, 1942)

THE EXPERIMENTS to be described represent an attempt to determine (i) the functional structure of that portion of the spinal mechanism utilized by long spinal reflexes, and (ii) the kinetics of central activity involved in long spinal reflexes.

Cats were used throughout the present experiments. The animals were made spinal by transection accomplished through the dorsal atlanto-occipital membrane. Artificial respiration was instituted and the ether anesthesia discontinued. To provide the afferent limb of the long spinal reflex, the brachial plexus was approached from the dorsum, crushed distally and equipped with stimulating electrodes. An afferent volley, resulting from a single stimulus delivered through the electrodes on the brachial plexus reaches the spinal cord by the lower cervical and first thoracic dorsal roots. This generally accepted statement has been confirmed by observing the cord potentials evoked at various levels by a volley of this kind. Figure 1, constructed after the manner of Hughes and Gasser (1, Fig. 1), represents plots of (i) the intramedullary spike potential (dots) and (ii) the negative intermediary potential (circles) at appropriate levels. The greatest influx of activity occurs in the lower three cervical segments. The skew deviation in the distribution of the intramedullary spike potential reflects the heavy contribution made by the ascending collaterals which form the fasciculus cuneatus (cf. also 9). The "tail" directed caudally undoubtedly represents conduction in the shorter descending collaterals. Van Gehuchten has stated (11) that in the rabbit the descending collaterals of the first thoracic segment extend as far as the eighth thoracic segment.

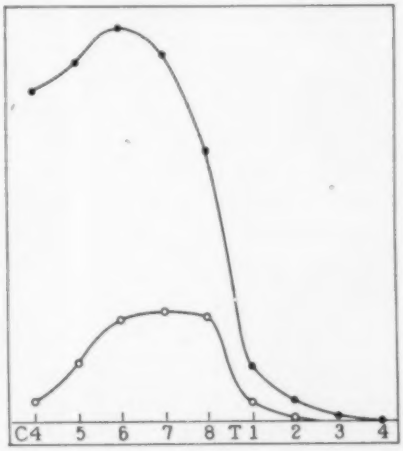

FIG. 1. Magnitude of intermedullary spike potentials (dots) and corresponding negative intermediary potentials (circles) evoked by a brachial plexus shock and plotted at various levels of the cervical enlargement.

Activity evoked in the cervical enlargement by a single brachial plexus volley has, in addition to spatial and intensity characteristics, a duration factor. The duration of the negative intermediary potential in this situation

* A preliminary account of some of the present results was presented at the meeting in Boston of the American Physiological Society (5).

is estimated by Therman (9) to be *ca.* 5 msec. It is known from analogous situations that internuncial activity, consequent upon dorsal root volleys, persists somewhat longer; in fact, no definitive statement relative to the duration of internuncial pool activity can be offered at the present state of knowledge, for various nuclei, subsequent to activation by an afferent volley, become silent at different times and in a roughly asymptotic manner.

In general it may be stated that vast pools of interneurons within the cervical enlargement become active for a period in excess of 5 msec. as the result of a single brachial plexus volley. It is the impulse output from these

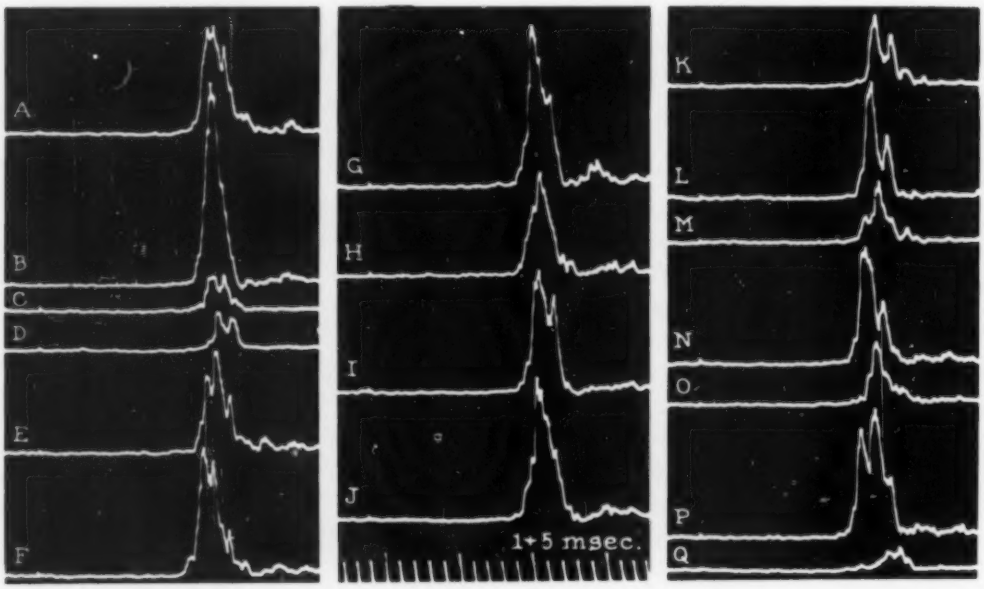

FIG. 2. Discharge of L7 motoneurons in response to an afferent volley in the brachial plexus. The records (all similar) were obtained with the use of ventral root leads and illustrate the variability of motor response.

active pools, directed caudally through the propriospinal system, that forms the input to the region under observation—the lumbar enlargement. Once below the caudal limit of active descending primary afferent collaterals, the responses to be considered pertain wholly to the intrinsic spinal system and to the motoneurons influenced by activity within that system. Thus in addition to the specific problem of the organization of long spinal reflex pathways, some of the present experiments have more general interest, inasmuch as they reveal features in the activity of pools of neurons when brought into play not by a synchronous nerve, root or tract volley, but by the diffuse, dispersed discharges projected from such other pools of neurons.

Records of the activity of the lumbar enlargement have been obtained, as in previous experiments (2, 4), by the use of microelectrodes placed at will within the cord, and by leads placed on selected ventral roots. Local

reflexes, occupying arcs of two neurons, have been employed to test the average excitability of selected motoneuron pools.

Long spinal reflex discharge. Figure 2 shows a series of long spinal reflex responses recorded in succession at 10 sec. intervals. In each case the afferent volley was provided by a standard brachial plexus shock; the efferent motor volley was recorded from the seventh lumbar ventral root (L7V.R.). One outstanding feature of the long spinal reflex discharge is its great variability, although variability in discharge is not unknown in even the simplest of spinal reflex arcs. Despite the intensity variation, however, the latency of the discharge is maintained relatively constant from one observation to another. In the 17 observations of Fig. 2 latency varies between 9.5 and 11 msec. A simple picture of the relationship between latency and intensity of the long spinal reflex discharges may be gained from the action potentials superimposed by the drawing in Fig. 3. Further examples to illustrate latency of the long spinal reflex discharge may be found in Fig. 4, 12, 13.

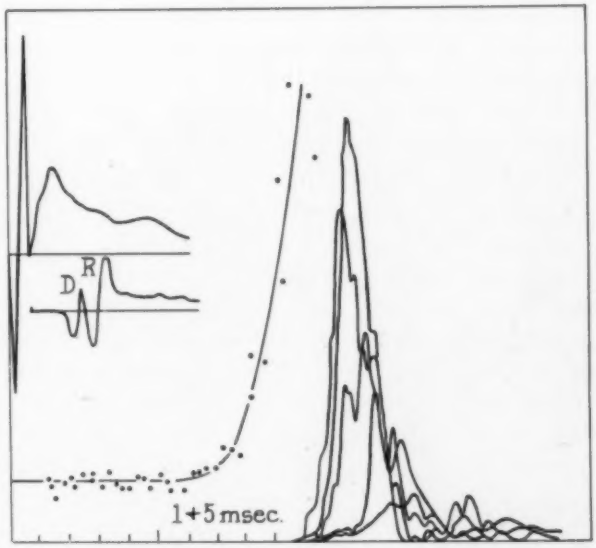

FIG. 3. Factors contributing to latency of long spinal reflex discharges. Top left: Cord potential evoked in the cervical enlargement by a brachial plexus volley. Next in order: Tract spike potentials recorded by microelectrode in the ventral column of the L7 segment in response to a ventral column shock delivered at the level from which the cord potential above is recorded. D, directly conducted tract spike potential. R, relayed tract spike potential (2). The tract spike potentials are drawn so that the shock artefact coincides in time with the onset of the negative intermediary potential above. Next in order: Graph showing onset of facilitation among L7 motoneurons as tested by a segmental two-neuron-arc discharge. Lower right: The discharge of L7 motoneurons. Five responses, including the extremes, are superimposed by drawing to illustrate variation in latency and intensity of the reflex discharge.

It may be noted that these experiments illustrate the extreme values for latency that have been encountered. The reflex latency is thus not only quite constant on successive observations on a single preparation; it is only slightly less constant from preparation to preparation. A further point of interest is the degree of synchrony with which the lumbar or sacral motoneurons discharge in response to a single brachial plexus shock. The discharge rarely outlasts 10 msec. and frequently is nearly complete in less than 5 msec., this in spite of the fact that internuncial impulses are contributed to the motoneurons and may be demonstrated as having a

facilitatory action there for a period in excess of 30 msec. (Fig. 15, 16). In summary then, the long spinal reflex discharge may be almost completely absent (Fig. 11), or it may approximate or even exceed (Fig. 12, 13) in size and synchrony the discharge obtained through short spinal reflex pathways. The latter fact is the more remarkable since as many as twenty spinal segments intervene between the afferent and efferent limbs of the long spinal reflex.

The course of events during the latent period for long spinal reflex discharge. The minimum reflex time for the long spinal reflex discharge approximates 9 msec. Figure 3 illustrates some of the factors to be considered in accounting for the observed reflex time. Assuming a single synaptic relay in the cervical enlargement between primary afferent fibers and long propriospinal neurons, and direct activation of some long propriospinal neurons by primary afferent impulses, the first impulses to reach the L7 segment would do so at *ca.* 2.5 msec. after the causal brachial plexus shock. This value is determined in the following manner. The cord potential of the cervical region evoked by a brachial plexus volley is recorded. This potential is drawn as the first record in Fig. 3. The onset of the negative intermediary potential signals the first internuncial activity resulting from the primary afferent volley. Stimulating electrodes are placed in the ventral column at the level previously used to record the cervical cord potential. A microelectrode is placed in the ventral column at the L7 segment and the tract spike potentials evoked by the ventral column shock are recorded as detailed in an earlier paper (2). For Fig. 3 the tract spike potentials of the direct (D) and relay (R) neurons are drawn in such a way that the stimulus artefact of the tract volley coincides in time with the onset of the negative intermediary potential of the cervical cord potential record above. The beginning of negativity at the microelectrode inserted into the ventral column measures the time at which, after the brachial plexus shock, the first propriospinal impulses might be expected to enter the L7 segment.

The value of 2.5 msec. must be taken as representing an order of magnitude, not a definitive value. In the first place the time at which the negative intermediary potential begins almost certainly marks the onset of internuncial activity in the dorsal horn. The synaptic delay indicated by this potential ranks among the shortest known. The largest propriospinal fibers characteristized by the highest conduction velocities might reasonably be supposed to convey the first impulses to the lumbar cord. These fibers belong to the ventrolateral columns (10) and undoubtedly have their cells of origin in the ventral horn. The synaptic systems of the ventral horn which have been adequately studied (primary afferent collaterals to motoneurons [6]; ventral column collaterals to short propriospinal neurons of the ventrolateral columns, and to motoneurons [2]) are characterized by somewhat longer delays in the "resting" cord. It is thus not unlikely that the longer delays should be allowed for the primary afferent-long propriospinal synaptic system. Secondly it is obvious that stimulation of the ventral columns activates descending systems other than purely propriospinal (2), with the implication that the ventral column record reveals predominantly vestibulospinal or reticulospinal activity. The force of the latter objection is mitigated by the fact that essentially similar records have been obtained in chronic spinal cats from whose cords extrinsic descending mechanism was eliminated by degeneration (unpublished observations). With due regard to these considerations, a value of 2.5 to 3.0 msec. is a fair and just approximation for the time of arrival of impulses in the L7 segment, given a single relay at the cervical level.

Facilitation of the lumbar motoneurons begins 6 to 7 msec. after the brachial plexus shock. The graph of Fig. 3 shows the early part of the facilitation curve of lumbar motoneurons, obtained by plotting the amplitude of a segmental two-neuron-arc discharge as a function of the interval by which the segmental test shock follows the conditioning brachial plexus shock. Between the delivery of impulses into the motor nucleus, as measured by facilitation therein, and the discharge of impulses from that nucleus there is, under the conditions imposed by long spinal reflex activation, a nuclear

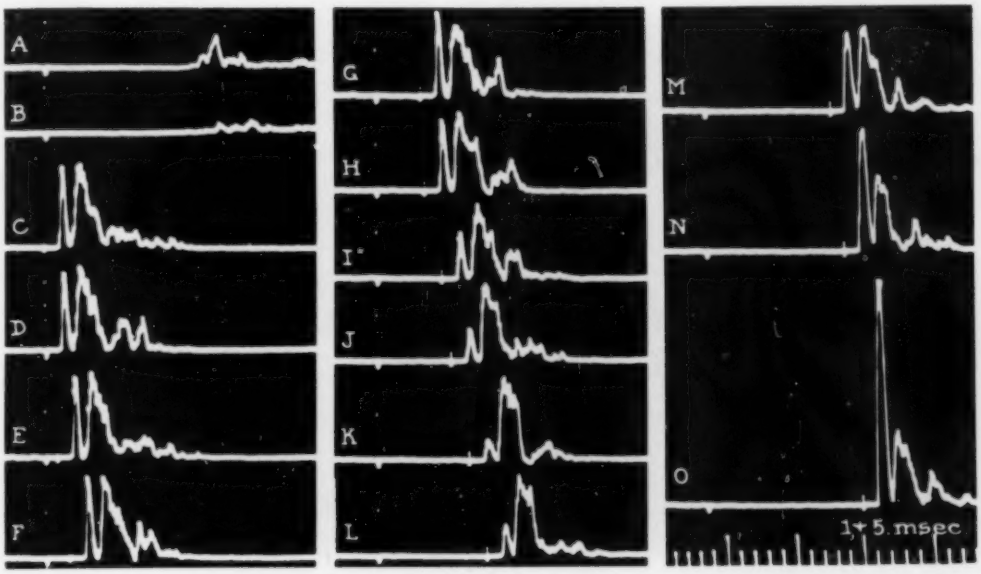

FIG. 4. Direct inhibitory action of propriospinal (internuncial) impulses on motoneurons as indicated by two-neuron-arc test reflex. A and B, response of L7 motoneurons to long spinal reflex activation. C, response of L7 motoneurons to segmental reflex activation. D-O, the segmental reflex tests the excitability of certain motoneurons during the latent period of the long spinal reflex. Note inhibition of the two-neuron-arc discharge, increasing progressively until the shock interval in K is attained; thereafter recovery and facilitation.

delay* of 2 to 3 msec., due allowance of course being made for ventral root conduction. It is clear from Fig. 3 that nuclear delay at the motor nuclei does not account for the total time between the hypothetical onset of tract activity in the L7 segment and the discharge of impulses from the motor nuclei. There remains an interval of 4 to 5 msec. preceding the onset of motoneuron facilitation for which some accounting must be made.

Direct inhibition. On many occasions when a segmental two-neuron-arc reflex discharge is utilized to measure the excitability of lumbar motoneurons following a brachial plexus shock, the first excitability change to occur is

* Nuclear delay is defined as an observed discrepancy in time between the arrival of impulses in a nucleus and the discharge of impulses from that nucleus under specified conditions of excitation.

not facilitation at 6 to 7 msec. after the shock, as in Fig. 3, 5 B, 5 C, but inhibition occurring with a latency of 2.5 to 3 msec. Figure 4 presents the results of such an experiment. Observations A and B of Fig. 4 illustrate the response of L7 motoneurons to single brachial plexus shocks, while 4C illustrates the response to a single L7 dorsal root (D.R.) shock. In Fig. 4 (D to O) the segmental reflex is introduced at increasing intervals after the brachial plexus shock, in order that the two-neuron-arc discharge may form a test of the excitability of certain motoneurons. In records D, E, F, and G no change occurs other than variation. From G to K, however, inhibition of the two-neuron-arc discharge occurs, increasing progressively, after which (L to O) inhibition is supplanted by facilitation. Graphs, further illustrating

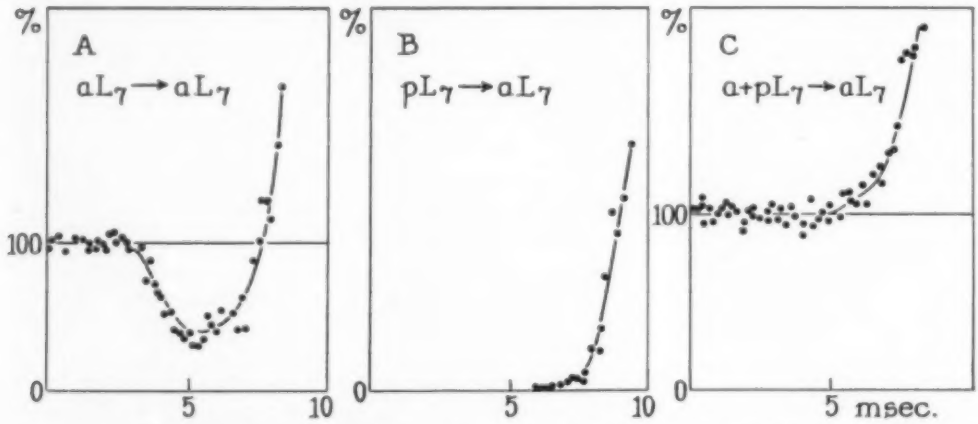

FIG. 5. "Masking" of inhibitory action. The effect of long spinal reflex activity on motoneurons of the anterior half of the L7 segment (aL7) is tested in A by the two-neuron-arc reflex evoked by a volley in the anterior half of the L7 dorsal root; in B, by the reflex evoked by a volley in the posterior half of the L7D.R.; and in C by the reflex evoked by stimulation of the whole dorsal root. The inhibition revealed by curve A is concealed in curve C. Further description in text.

the time course of inhibition in other experiments, are presented in Fig. 5A, 15, 16.

It is noteworthy that inhibition begins at the time predicted for the arrival of the first long propriospinal impulses within the L7 segment. A demonstrable excitability change in the motor nucleus at this time interval is powerful evidence that the propriospinal impulses do in fact arrive at the predicted time. The present example of inhibitory action is entirely analogous to that previously found to occur in short spinal reflex systems (3), with the important exception that the inhibition in this case is clearly attributable to the action of interneurons.

The masking of inhibitory activity. It is apparent from a comparison of Fig. 3 and 4 that all two-neuron-arc reflex discharges do not behave in a similar manner when brought into conjunction with long spinal reflex activity. Facilitation has always been observed as shown in Fig. 3, 5, 15, 16, but the earlier inhibition period is not infrequently absent. It might be

supposed that this fact indicates merely that some motoneurons receive inhibitory action coincident with the arrival of propriospinal impulses, whereas others do not. Some experiments have shown, however, that the fact may not be fully explained in this simple fashion. The results described in connection with Fig. 5, for instance, illustrate how an inhibitory action may be masked, presumably by virtue of some interaction within the testing system.

For each of the curves of Fig. 5 a standard brachial plexus volley provides the conditioning activity, and in each case the tested motoneurons lie within the pool contributing axons to the anterior half of the L7V.R. For curve A the test shock is applied to the anterior half of the L7D.R. and results in a two-neuron-arc reflex discharge, the amplitude of which is plotted in curve A as a function of the interval between the conditioning and testing shocks. In curve A inhibition begins *ca.* 3 msec. after the brachial plexus shock, and reaches a maximum between the 5th and 6th msec., after which facilitation supervenes.

Curve 5B is constructed in a manner similar to that of 5A. The test shock, however, is applied to the posterior half of the L7 D.R. There is no two-neuron-arc discharge in the control test responses, but it does appear by virtue of facilitation after a shock interval of *ca.* 6 msec. Since the two-neuron arc is subliminally active in the control responses, this test cannot indicate the presence or absence of an inhibitory period like that observed in 5A.

If now the whole L7D.R. be stimulated, a two-neuron-arc reflex response results, which is somewhat greater than that resulting from stimulation of the anterior half of the L7D.R. alone, due to convergence at the motoneurons of the primary afferent impulses of the two halves of the dorsal root. Curve 5C shows the effect of the long spinal reflex impulses as tested by the two-neuron arc evoked by stimulation of the whole L7D.R. No demonstrable excitability change occurs antecedent to the onset of facilitation in the 6th msec. Therefore, an interaction has taken place between the anterior and the posterior reflexes employed severally as tests in 5A and B respectively, on the occasion of their simultaneous combination. As a result, the inhibitory action of the long spinal reflex is either counteracted or masked.

Two interpretations of these observations seem worthy of consideration, although others undoubtedly could be developed. The choice between the interpretations depends upon whether one is to assume that the motoneurons responding after a single synaptic delay to stimulation of the anterior half of the L7D.R. alone do so when the whole dorsal root is stimulated, or conversely do not do so. Either assumption is reasonable. If motoneurons contributing to the test discharge employed in 5A also contribute to the test discharge of 5C, then it would seem that the convergence of anterior and posterior L7 impulses at the motoneurons, while not recruiting many more motoneurons into the test discharge (test response C is not much greater than test response A) has so fortified the synaptic drive within the firing zone that the inhibitory action of the long spinal reflex impulses is completely

overcome. Alternatively the posterior L7 dorsal root volley may be regarded as recruiting motoneurons heavily from the subliminal fringe of the anterior L7 reflex, the motoneurons so recruited not belonging to the fraction of

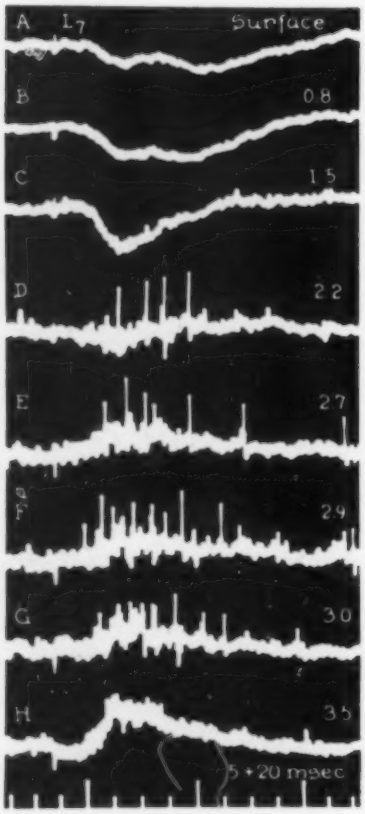

FIG. 6. Long spinal reflex. Activity recorded by microelectrode in the L7 segment. The figures at the right give the depth of the recording tip below the surface. Note the slow potential positive in the dorsal regions and negative in the ventral regions of the cord, also localization of the active neurons revealed by the occurrence of spike potentials.

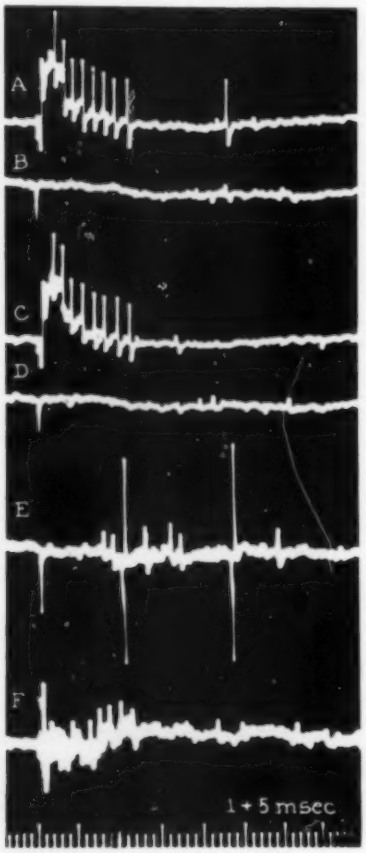

FIG. 7. Records obtained by microelectrode in the L5 dorsal (A, B, C, D) and ventral (E, F) horns. A, C and F responses to L7D.R. volley. B, D and E responses to brachial plexus volley. Convergence occurs only in the ventral horn. Further description in text. The two long spike potentials in E may represent motoneuron activity.

the pool subject to inhibition by the long spinal reflex impulses. At the same time, by direct inhibitory action of its own, the posterior L7D.R. volley must remove from the responsive system those motoneurons that contribute to the test response employed in 5A, and which can be inhibited by the action of the long spinal reflex impulses. Whatever may be the unique explana-

tion of observations such as those of Fig. 5, it is well to remember that the absence of any change in a two-neuron-arc reflex test does not preclude the occurrence of inhibitory action by the conditioning activity. The occurrence of an inhibitory action can be denied, and then with some reservations, only after an exhaustive search has been made with uniformly negative results.

Responses of the gray substance. Following a single shock to the brachial plexus and the resulting discharge into the descending propriospinal system, certain pools of neurons within the lumbar enlargement become active. The pools of active neurons are almost, if not entirely, confined to the ventral horn.

Figure 6 shows a series of records made at various depths in the dorsoventral axis of the spinal cord extending from the dorsum to the ventral margin of the ventral horn. The recording microelectrode was inserted from a point 1 mm. medial to the root entry line and therefore passes just medial to the heart of the gray substance. Records A, B and C of Fig. 6 present typical results obtained when the microelectrode tip lies on the dorsal surface, in the dorsal column or dorsal horn. The dorsal regions of the cord become positive as a result of the brachial plexus shock, for a period of time approximately coextensive with the facilitation period of the motoneurons (cf. Fig. 15, 16). The neurons of the dorsal horn do not become active, a fact which has repeatedly been confirmed. For example, in Fig. 7 dorsal horn units of the L5 segment are seen to respond in A and C to a single L7-D.R. shock. These dorsal horn units, however, are quiescent during the activity period resulting in B and D of Fig. 7 from a brachial plexus shock. The dorsal horn cannot, therefore, be concerned in a primary sense with long spinal reflexes. Occasionally, if the hind limb movement caused by the long spinal reflex action be great, a small burst of dorsal horn activity may result. When this occurs, it does so with a latency of approximately 30 msec., which fact clearly defines it as a rebound from the periphery.

As the recording microelectrode passes from the intermediate region through the ventral horn, nuclear discharges are written on a slower negative potential which appears to increase in amplitude as the microelectrode approaches the ventral margin of the ventral horn. When the margin is reached, the nuclear discharges fade out, leaving the slower negative potential in prominence (Fig. 6H). Units within the ventral horn that are open to activation by the sequelae of a brachial plexus shock may also be available to local dorsal root volleys. An example of this observation is presented in Fig. 7, in which E shows the discharges of two types of units in response to a brachial plexus volley, while F reveals the smaller units unmistakably responding again as the result of a local dorsal root shock. These experiments indicate that whatever convergence of long and short spinal reflexes occurs, must take place within the ventral horn.

The more lateral regions of the ventral horn are also active following brachial plexus stimulation. Figure 8 (A to G) presents records obtained in the lateral part of the ventral horn of the L5 segment. The microelectrode

is placed at successive positions from 2.5 to 3.1 mm. below the dorsal surface of the cord. Figure 8H was recorded in the L6 segment, 3.2 mm. below the dorsal surface and 0.7 mm. lateral to the root entry line, to demonstrate the

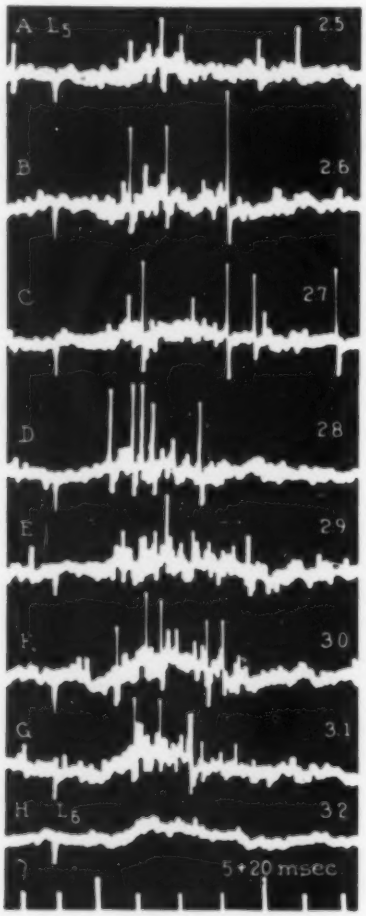

FIG. 8. Long spinal reflex activity recorded from the lateral part of the ventral horn in the L5 segment. The figures at the right give the depth of the recording tip of the microelectrode. H, record lateral to the ventrolateral angle of the ventral horn in the L6 segment.

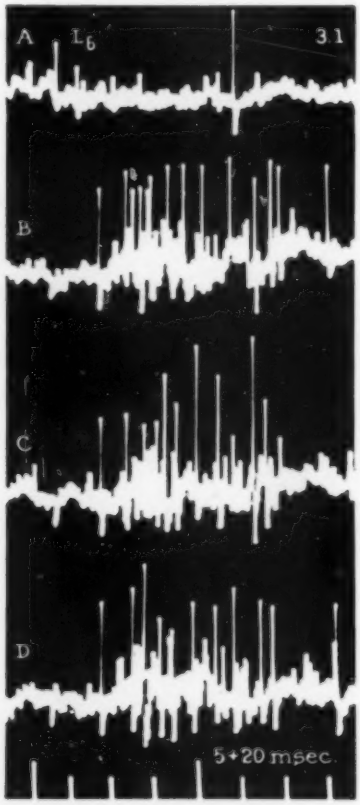

FIG. 9. Records from the medial aspect of the ventral horn in the region of the *nucleus cornu-commissuralis anterior*. A, blank sweep. B, C, D, all similar.

presence of the slower negative potential at the lateral margin, as well as at the ventral margin of the ventral horn.

The most prominent discharges to be found in the lumbar spinal cord, on stimulating the brachial plexus, occur at the medial aspect of the ventral horn. The records of Fig. 9 were all obtained in the L6 segment, 1.4 mm. medial to the root entry line and 3.1 mm. below the dorsal surface. Observa-

tion A shows the level of activity maintained in the absence of specific stimulation, whereas B, C, and D (all similar) illustrate the profusion of activity elicited by a single brachial plexus shock. These powerful discharges are of particular interest, for they are found characteristically in this area heavily endowed with commissural neurons (*nucleus cornu-commissuralis anterior*). That the activity of commissural neurons is so conspicuous will not be occasion for surprise when the extent to which crossed activity participates not only in contralateral reactions, but also in "purely ipsilateral" reactions is appreciated (cf. Fig. 15, 16).

In general it may be stated that the gray substance of the side contralateral to that receiving the afferent brachial plexus volley becomes active in a pattern entirely comparable to that which has been described for the ipsilateral gray substance.

With the examples of nuclear activity presented in Fig. 6, 7, 8 and 9 considered together, certain conclusions may be reached. Virtually all regions of the ventral horn exhibit activity as a result of brachial plexus stimulation, whereas the dorsal horn and most, if not all, of the intermediate region remain silent. This fact is worth noting in interpreting the sign of the slow potentials, such as those recorded in Fig. 6. These potentials are essentially similar in nature to the intermediary potentials discussed by Hughes and Gasser (1), with the outstanding difference that the polarity is reversed in the dorso-ventral axis. The reversed sign is to be correlated with the fact that, with brachial plexus stimulation, activity in the lumbar enlargement is centered in the ventral horn, while under the conditions of local afferent stimulation intense activity is engendered in the dorsal horn.

Nuclear activity begins 5 to 7 msec. after the afferent stimulus, as evidenced both by the slow potentials and by the earliest recorded spike potential activity. During the interval between the 10th and the 20th msec. after the shock, nuclear activity is most intense, following which reversion to the resting state occurs, the most prolonged discharges usually ceasing within 35 to 40 msec. The onset of nuclear activity, therefore, coincides with the beginning of facilitation in the motor pools, and the two events follow parallel subsequent courses. One may conclude that the local internuncial pools, rather than the tract fibers, are primarily responsible for excitation in the motor nuclei. It follows then that the tract fibers are rather more liberally distributed to the internuncial pools of the ventral horn than to the motoneurons, at least insofar as their end-action is excitatory.

On many counts the discharges illustrated in Fig. 6, 7, 8 and 9 represent preponderantly the activity of interneurons and not that of motoneurons. For instance, evidence may be found in the fact that unitary discharges such as illustrated precede by a distinct interval the onset of motoneuron discharge, while the subsequent time-intensity course parallels, not motoneuron discharge, but motoneuron facilitation as measured by test two-neuron-arc discharges. In some experiments in which motoneuron discharge is copious, this discharge appears at the microelectrode as a deep positive

potential cutting into the heart of the more prolonged negative potential. In other experiments the anatomical location of the microelectrode minimizes the possibility of motoneuron contribution to the record, as in Fig. 9. The size of the recorded spike potentials is in some measure a criterion, most of the spike potentials recorded in the present experiments being no more than several hundred microvolts in amplitude, whereas large neurons contribute spike potentials of 2 or more millivolts (cf. the solitary cells of the dorsal horn, [4]). Finally it is a common experience that unitary discharges referable to motoneurons are notoriously difficult to record, for the motoneurons are usually almost immediately damaged by the approaching microelectrode.

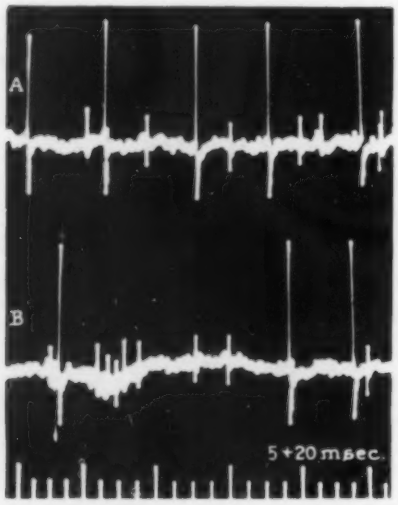

FIG. 10. Records from the ventral horn showing in A, the spontaneously occurring discharges of two units, one yielding the large spikes, the other small spikes. In B, the large spikes are suppressed, the small spikes are multiplied by long spinal reflex activity. The neurons yielding these spikes are reciprocally related under the conditions of long spinal reflex action.

On such occasions a furious injury discharge is encountered, the individual spike potentials of which are several millivolts in amplitude. On the contrary, the responses pictured in Fig. 6, 7, 8 and 9 are easily and regularly obtainable. It is possible that the two large spike potentials appearing in Fig. 7E represent motoneuron discharges, for they occur with appropriate latency and are of large size.

Inhibition at internuncial levels—reciprocal innervation. Neurons within the ventral horn are not always brought into activity subsequent to brachial plexus stimulation; occasionally neurons are found, by an exploring microelectrode, in which activity is suspended rather than initiated. For example, in Fig. 10A there are two types of units responding spontaneously and yielding characteristic recorded spike potentials. The microelectrode for these observations was placed 2.8 mm. below the dorsum, at the root entry line of the lower part of the L5 segment, *i.e.*, in the ventral horn. For record 10B a single shock was delivered to the brachial plexus. The responses identified by the smaller action potentials are strongly intensified, whereas the larger spike potential responses are suspended for a period in excess of 50 msec.

The two units clearly are reciprocally related, under the conditions imposed by long spinal reflex activation.

Interaction between long spinal reflexes and local reflexes of more than two neurons. Reference to Fig. 4 shows that the discharges through arcs of more than two neurons in the segmental reflex are not affected until the shock intervals used in records 4N and 4O are reached. There is then a decrease in the discharge pertaining to these multineuronal arcs. In the case of the segmental reflex it might be said that such a decrease in higher order discharges is due to occlusion by the simultaneously facilitated antecedent two-neuron-arc discharge. This explanation cannot be ruled out, as there

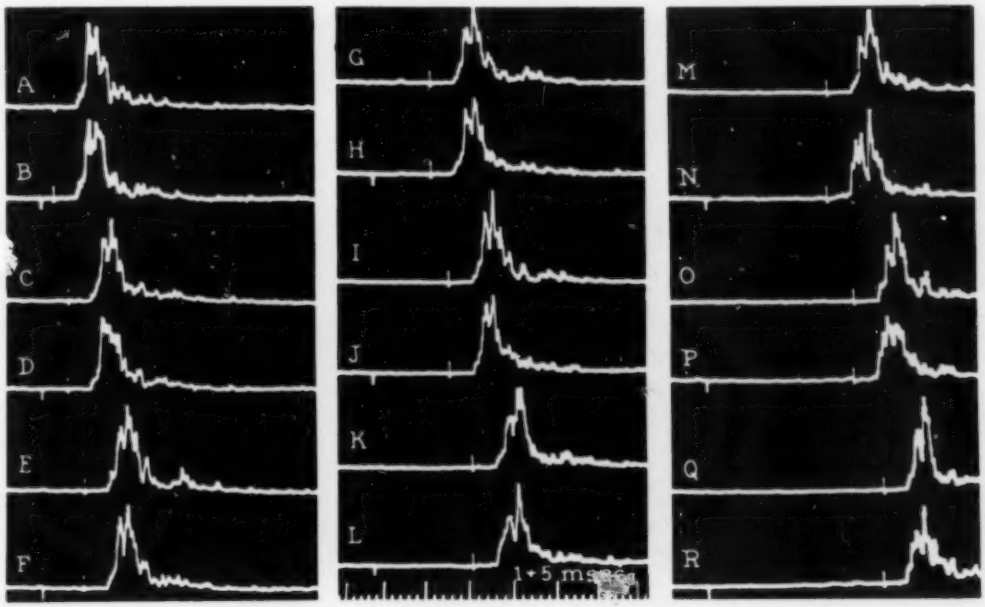

FIG. 11. Interaction of long spinal reflex and short spinal reflex having as its shortest path one of more than two neurons. Control records of the short spinal reflex (A, C, E . . .) are alternated with observations on interaction (B, D, F . . .). The motoneuron discharge evoked through the long spinal reflex paths is very small, but can be identified in L, N, P and R.

is every indication that the finer structure of the reflex system is such as to permit this to occur. To study internuncial interactions it is desirable to devise test reflexes unencumbered by two-neuron arcs.

In Fig. 11 a reflex consisting of the discharge elicited among S1 motoneurons by a L6D.R. shock is placed at various intervals during the course of the activity evoked by a brachial plexus shock. At no time are two-neuron arcs involved in the reactive systems, so that activity in the higher order arcs is not thereby obscured. The testing L6 to S1 reflex is somewhat variable. Accordingly controls are alternated with conditioned responses, so that a fair estimate of interaction may be attained. In this experiment, motoneuron discharge in response to the brachial plexus stimulation in

isolation is insignificant. No tangible interaction occurs before an interval of 14 msec. separates the two shocks (11N). At this interval the earliest discharges of the local reflex appear to be favored over the later discharges. It is known that internuncial activity and motoneuron facilitation begin about 6 to 7 msec. after the delivery of a brachial plexus shock. The mild facilitation of higher order arcs, seen in 11N and 11P, takes place only at the peak of the facilitation period of motoneurons as revealed by two-neuron arcs (at 15 to 20 msec., cf. Fig. 15, 16). It is most likely, therefore, that the higher order arcs are influenced under the circumstances at the final relay, although on the basis of the observations of Fig. 7 (E and F) some interaction at interneurons of the ventral horn may be expected. It is probable that the greatest part of the local reflex pathways relay in the dorsal horn

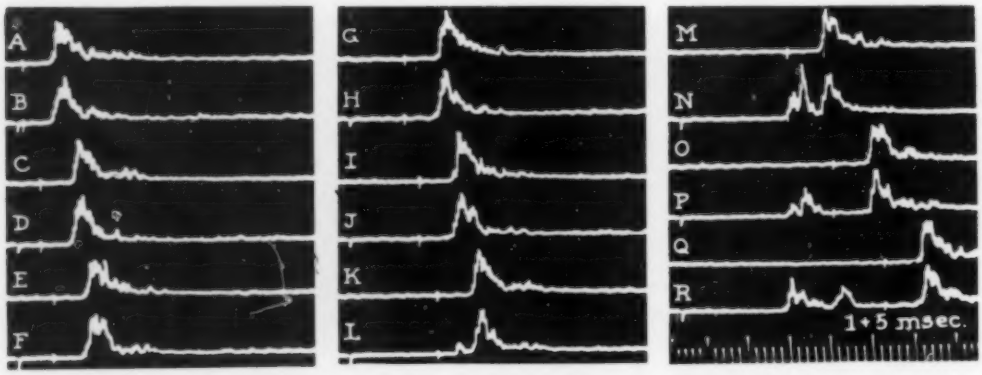

FIG. 12. Experiment similar to that illustrated in Fig. 11, but from another preparation in which the long spinal reflex discharge is ample. Note that the short spinal reflex obliterates the long spinal reflex discharge, but does not in turn suffer complete inhibition. The interaction between these two reflexes is asymmetrical.

and the intermediate nucleus of Cajal, rather than in the ventral horn, for if the converse were true one would expect to find significant changes in the short spinal reflex occurring long before they do, perhaps even as early as 2.5 to 3 msec. after the brachial plexus shock. It will be recalled in this connection that facilitation of local reflexes at the intermediate nucleus is the primary event when the pyramidal system forms the source of conditioning activity (4).

More striking than the allied or reinforcing actions of long spinal reflexes on local reflexes of higher order are the antagonisms between these two classes of reflex. In Fig. 11 it will be seen that the discharge area of the local reflex is slightly decreased in P and R as a result of the long spinal reflex activity.

Figure 12 presents additional features in the interaction of long and short spinal reflexes. It is constructed in the same way as Fig. 11, utilizing the same reflex pathways, but it is taken from another experiment in which the motoneuron response to the brachial plexus stimulus is of considerable

size. This response is seen in the first discharge grouping in Fig. 12R; the second grouping belongs to the short spinal reflex. It will be seen from observations A to J of Fig. 12 that the short spinal reflex discharge in simultaneous combination with (J), or preceding the long spinal reflex discharge, obliterates the latter. On the other hand, as the short spinal reflex falls later within the long spinal reflex discharge period (L and N) or follows after that period (P and R), it is in turn but little affected. The interaction between these reflexes then is far from symmetrical.

Observations of particular interest have been made in several experiments by causing the two reflex discharges to fall in simultaneous combination, as in Fig. 12J. The effect, to be described in greater detail in connection with Fig. 13, is the more striking when the long spinal reflex discharge is

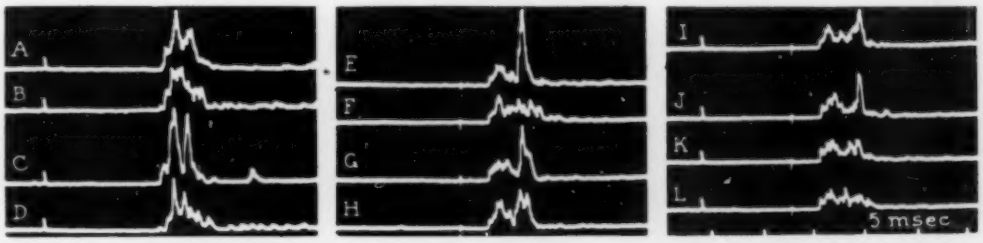

FIG. 13. Mutual inhibition of long and short spinal reflexes. The shock interval is adjusted so that the two reflex discharges fall in simultaneous combination. A–D, long spinal reflex. E–H, short spinal reflex. I–L, combined reflexes.

sizeable. In Fig. 13 are presented the results of one such experiment. A brachial plexus shock and an L6D.R. shock are set at such an interval that the motoneuron discharge periods of the two reflexes coincide in time. On account of the variability of response, four sets of responses to the shocks in isolation and together are reproduced. The shocks are separated by approximately 9 msec. Records A, B, C, and D of Fig. 13 show the response elicited by the brachial plexus shock alone, while E, F, G and H show the response to the L6 shock alone. In I, J, K and L the two shocks are delivered in succession at the predetermined interval. It is obvious that the response to the combined stimulation is much less than the sum of the responses to either reflex stimulation alone. What is more, all of the responses elicited by the two shocks in concert are smaller than any of the responses evoked by either of the two shocks in isolation. The effect illustrated in Fig. 13, therefore, is too great to be accounted for in terms of occlusion; there is actually a mutual inhibition between the two reflexes.

It is apparent in the observations of Fig. 11, 12 and 13 that the long spinal reflex has a feeble inhibitory action on the short multineuronal reflex discharge, and that this action is virtually independent of the size of the motoneuron discharge evoked through the long spinal reflex pathways. It is improbable, therefore, that motoneuron activity, as the final link of the long spinal reflex, is a factor contributing to the observed inhibition of the

short spinal reflex. It is known from Fig. 10 that some interneurons of the ventral horn are inhibited by the brachial plexus volley, and undoubtedly some motoneurons are inhibited for a similar period of time.

The evidence for direct inhibition is largely derived from conditioning curves such as those seen in Fig. 5A, 15, 16. The response to the test volley measures the average excitability of a selected pool of motoneurons. It does not and cannot reveal whether the motoneurons which are inhibited initially are subsequently excited, and contribute therefore to the facilitation phase of the conditioning curve; or whether they remain inhibited throughout, taking no further part in the cycle of events, while other individuals of the motoneuron population are recruited into the test discharge. Since the latter is manifestly true for interneurons (Fig. 10), there is no reason to suppose that it is not also valid for motoneurons.

It has been noted that the long spinal reflex discharge is relatively variable. When this discharge is smaller than that of the short spinal reflex, its obliteration by the short reflex might be explained by occlusion. However, in view of the asymmetry of interaction between the two reflexes (Fig. 12) and the magnitude of the inhibitory effect exerted by the short spinal reflex (Fig. 13), blocking as it does discharges greater than itself, occlusion cannot adequately account for the results. There is fortunately a good deal of evidence to show that dorsal root volleys through the L6D.R. exert a direct inhibitory action on motoneurons of the S1 segment as tested by two-neuron-arc discharges.

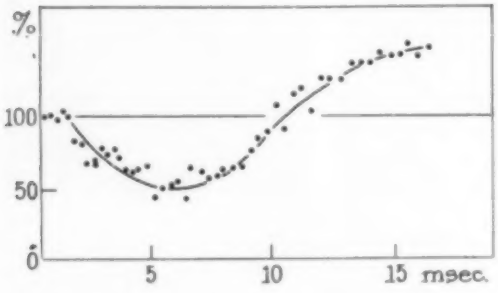

FIG. 14. Inhibition of some motoneurons of the S1 segment as evoked by L6 dorsal root volleys and tested by a two-neuron-arc discharge. The latency for inhibition in this example is *ca.* 1.5 msec.; other S1 motoneurons are facilitated with the same latency (cf. 3, Fig. 2). The motoneuron discharge of the S1 pool to L6 shocks occurs within the period during which these motoneurons are inhibited and the others are facilitated. Further description in text.

In the original description of direct inhibitory action (3) inhibition lasted for only 1 to 1.5 msec., thereafter being supplanted by facilitation. Although this is a frequent finding, some experiments by virtue of a different selection of testing two-neuron-arc discharges reveal that inhibition of some motoneurons within the S1 pool is maintained throughout the time during which others are discharging in response to the L6 volley. Figure 14 illustrates an experiment of this type. Amplitude of the two-neuron-arc test discharge is plotted as a function of the interval between the conditioning and the testing shocks. The motoneurons tested belong to the S1 segmental pool. No change in the test discharge appears during the first 1.5 msec., although "masking of inhibition" may account for this. For the next 10 msec., during which a few S1 motoneurons are discharging, others involved in the two-neuron-arc test are inhibited. The effect illustrated in Fig. 14 would account for the

suppression of the long spinal reflex discharge, given an appropriate selection of motoneurons by the long spinal reflex.

The interaction between long and short spinal reflexes, as presented in Fig. 11, 12 and 13 is one of considerable complexity. It seems certain that the results of these experiments represent in large measure the interplay of direct inhibitory actions. The two reflexes, long and short, are preponderantly though not exclusively antagonistic. The antagonism, moreover, is not basically one of competition for pathways; indeed, the present experiments point to a rather high degree of independence between the paths occupied by the two reflexes, at least insofar as excitatory actions are concerned. Until the mechanism of direct inhibition and the substrate of finer

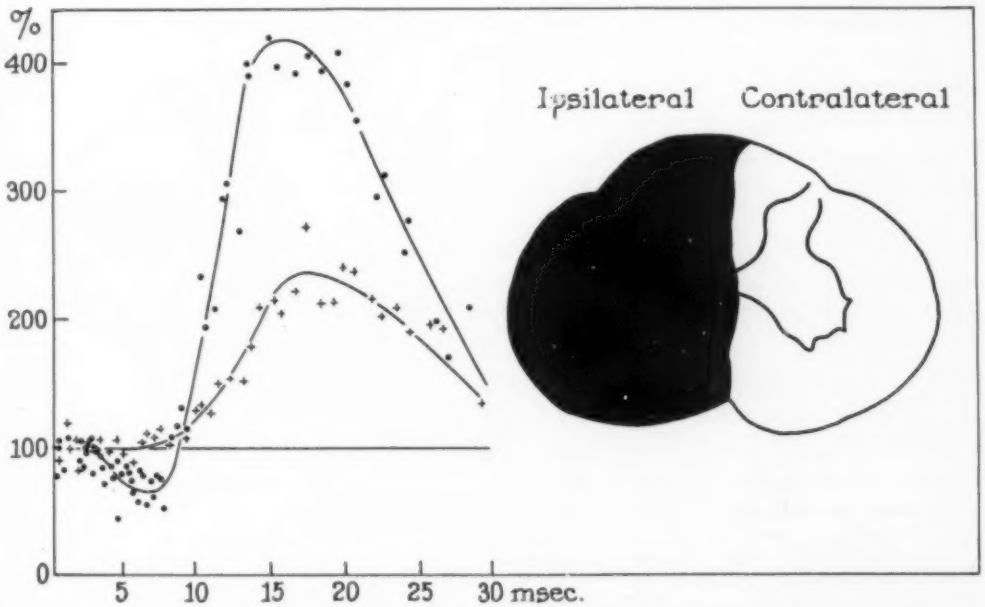

FIG. 15. Effect of ipsilateral hemisection in the upper lumbar cord on inhibition and facilitation of lumbar two-neuron-arc discharges by brachial plexus volleys. On the left are conditioning curves obtained before (dots) and after (crosses) hemisection. At the right is shown the extent of the lesion as determined from serial sections.

anatomy underlying that form of inhibition are better understood, no statement on inhibitory convergence can have more than the chance validity of an *ad hoc* argument.

The interaction between long spinal reflexes is quite different from that between long and short spinal reflexes. In brief, facilitation of motoneuron discharge is the final result, whether the successive long spinal reflexes both originate from the same side of the body, or whether they arise from opposite sides.

Tracts mediating long spinal reflex activity—effect of partial lesions of spinal cord. The impulses which link the cervical and lumbar enlargements during the course of long spinal reflex activity are so diffuse and dispersed that the

direct experimental approach by the use of microelectrodes has failed to yield significant results. These have been obtained, however, by resorting to a study of the effect of spinal cord lesions upon the course of facilitation and inhibition of lumbar motoneurons by brachial plexus shocks. The discharge of motoneurons has not been used because of the variability of response.

A hemisection, ipsilateral with respect to the brachial plexus stimulation and the tested motoneurons, abolishes the early inhibitory period, but does not similarly abolish the period of facilitation. Figure 15 illustrates this finding. On the left of Fig. 15 are drawn the conditioning curves of a segmental two-neuron-arc discharge, amplitude of the test response being plotted as

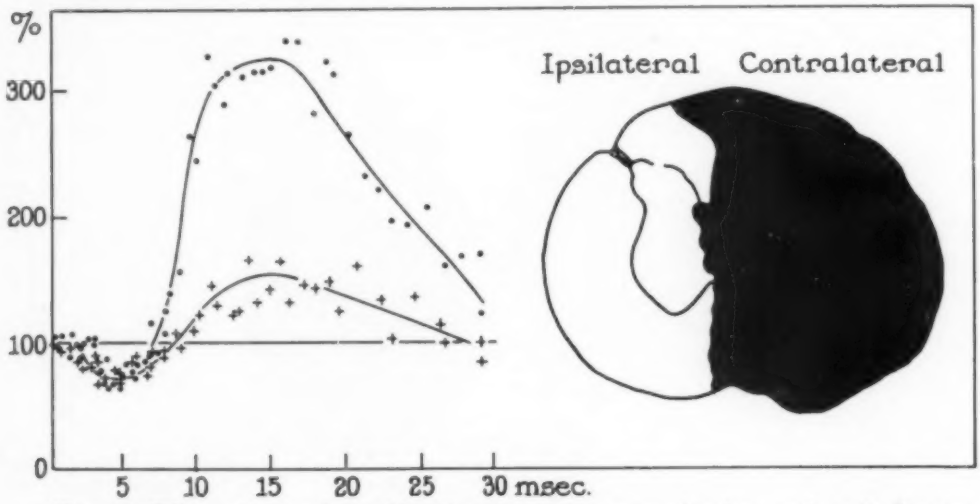

FIG. 16. Effect of contralateral hemisection in the upper lumbar cord on facilitation of lumbar two-neuron-arc discharges by brachial plexus volleys. Note that inhibition is not impaired by contralateral hemisection. On the left are conditioning curves obtained before (dots) and after (crosses) hemisection. The diagram at the right illustrates the extent of the lesion.

a function of the interval by which the test shock follows the brachial plexus shock. The curve represented by dots is constructed from observations obtained before hemisection, that represented by crosses from observations following hemisection. The extent of the lesion is shown on the right, as determined from serial sections. The drawing is made by projection, and the full extent of the lesion is found by recording the limits of the lesion as they occur in various sections. The lesion in Fig. 15 is actually slightly greater than a hemisection, as it transgresses the mid-line in the dorsal column and at the base of the ventral column. A comparison of the curves before and after ipsilateral hemisection shows that the paths mediating the direct inhibition are severed, whereas only a fraction of the excitatory paths is blocked.

Figure 16, constructed in the same way as Fig. 15, illustrates the effect of contralateral hemisection. The lesion in this case is complete on the contralateral side, includes about a third of the dorsal column on the ipsilateral

side, and damages some fibers close to the ventromedian fissure on the ipsilateral side. Other experiments (Fig. 17) have shown that involvement of the dorsal column is without significance, so that for practical purposes this lesion may be considered as a hemisection. Consideration of the conditioning curves in Fig. 16 shows that inhibition is not affected by contralateral hemisection, while facilitation is severely depleted although not abolished.

The results of Fig. 15 and 16 taken together indicate that the fibers mediating inhibition enter the lumbar enlargement with strict regard for laterality. On the other hand, the pathways mediating excitatory effects arising on one side of the body and exerting their action, under the conditions of the experiments, on that same side of the body, are not only ipsilateral and uncrossed, but are doubly crossed as well.

Similar effects are noted when the object of study is the crossed long spinal reflex. Thus lesions either ipsilateral or contralateral with respect to the stimulated brachial plexus deplete the facilitation of contralateral (crossed) two-neuron-arc discharges. Despite a number of attempts to uncover direct inhibition of two-neuron-arc discharges on the side contralateral to the stimulated brachial plexus, this has not been observed. It is believed not to occur.

The fact that the intraspinal pathway mediating direct inhibition is strictly unilateral (Fig. 15, 16), taken together with the observations on latency of the direct inhibitory action (Fig. 4, 5, 15, 16), confirms the view that the long propriospinal fibers themselves exert the inhibitory action. It will be remembered in this connection that Sherrington and Laslett (8) found no evidence to suggest that the long propriospinal fibers decussate. There seems to be no reason *a priori* why long propriospianl fibers arising in the cervical enlargement on the side contralateral to a given brachial plexus volley should not be activated by commissural neurons intrinsic to the cervical enlargement. Subsequently, and maintaining laterality, these propriospinal fibers could achieve an inhibitory effect on lumbar motoneurons on the side contralateral to the brachial plexus volley. The evidence on hand that contraindicates such an eventuality as this is of necessity inconclusive.

Lesions confined to the dorsal columns, of which one example is illustrated in Fig. 17A, do not result in a deficit in facilitation of two-neuron arcs in the lumbar enlargement. This is taken to indicate that aborally directed fibers of the dorsal columns do not participate to a significant degree in mediating long spinal reflex activity under the present conditions of experiment. Superficial lesions of the ipsilateral lateral column (Fig. 17B) have but a slight effect. When the ipsilateral lateral column is more seriously involved (Fig. 17C) a serious deficit in facilitation follows. Hence the ipsilateral column, and especially its deeper parts, is important for the mediation of ipsilateral excitatory effects.

The contralateral half of the spinal cord is important in mediating "ipsilateral" reflex effect, and the contralateral ventral column appears to contain

the majority of the fibers contributing to "doubly crossed" excitation. For instance, Lesion 17H, severely damaging the contralateral lateral column, is without effect on the ipsilateral reflex excitation. Comparison of Lesion 17C and Lesion 17H, these being virtually symmetrical lesions, leads to the conclusion that the lateral column is in the main reserved for truly unilateral ipsilateral transmission, *i.e.*, the intrinsic fibers of the lateral columns do not measurably supply the commissural nuclei. In this connection it is of interest

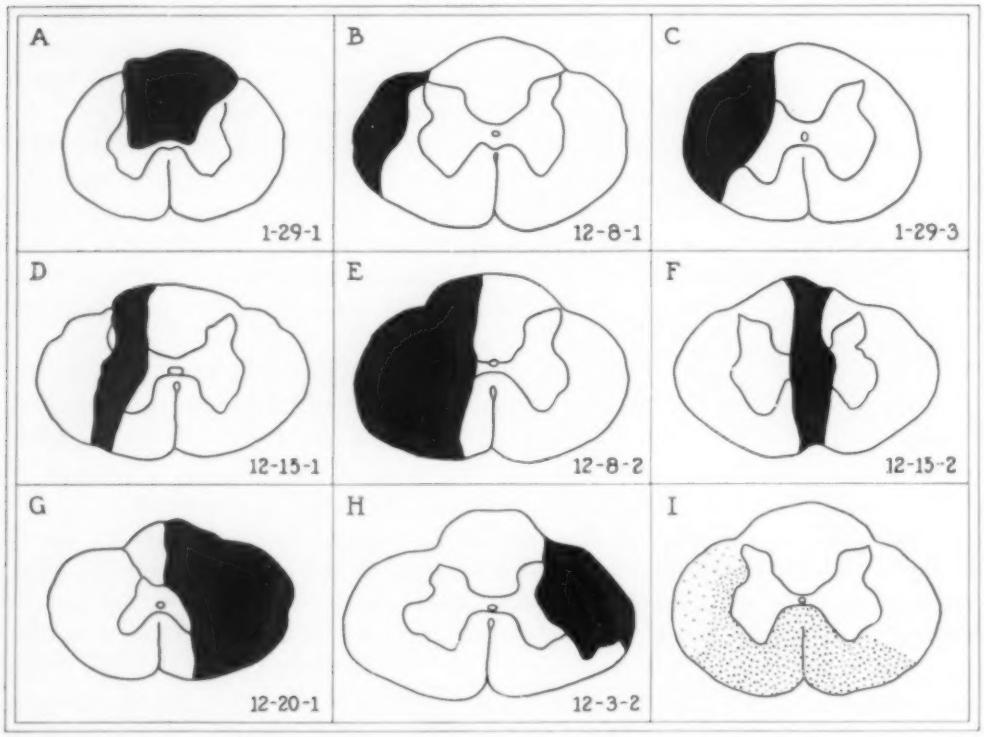

FIG. 17. A–H, various lesions of the spinal cord the effect of which on long spinal reflex action is discussed in the text. Lesions C and H are virtually symmetrical as are E and G. I, diagram of the cross-sectional area of the cord. The shaded area contains the tract fibers, ipsilateral and contralateral, which contribute to "purely ipsilateral" end effect. In essence the lateral columns carry unilateral tracts, the ventral columns bilateral tracts.

to recall the important part played by the lateral column in such exquisitely unilateral reactions as the scratch reflex (8).

All lesions transgressing on the ventral columns, ipsilateral or contralateral diminish the intensity of long spinal facilitation in varying degree (Lesions 17D, E, F, G). Given approximately symmetrical lesions of the ventral columns (Lesions 15 and 16, Lesions 17E and 17G), the contralateral lesion appears to violate facilitation to the greater extent. This observation may depend upon the fact that the inhibitory actions of brachial plexus volleys are pre-eminently ipsilateral. Accordingly, contralateral lesions would de-

plete only excitatory barrage mediated through commissural neurons, while ipsilateral lesions would diminish inhibition along with excitation. In the former case the residual excitatory barrage might be pitted against the full inhibitory power of the reflex activity; in the latter case the contralateral barrage would be relatively unfettered by inhibition.

Diagram I of Fig. 17 illustrates the areas contributing descending fibers for the transmission of ipsilateral long spinal reflex excitatory effect. It is constructed according to the information gained from experiments involving lesions, including those illustrated. Emphasis must be placed on the view that the present experiments, in which long spinal reflex pathways are activated in mass, tend to delineate the overall dimensions of the system, rather than the dimensions of the system as utilized for the performance of any appropriate reflex act. Obviously, localizations within the system exist for the transmission of impulses involved in the performance of certain acts, the scratch reflex being a case in point. In fact, some localization is revealed in the present experiments, inasmuch as the cross-sectional area of the cord contributing fibers for the realization of unilateral end effect is asymmetrically bilateral, whereas the fasciculi proprii of the cord considered as a whole are bilaterally symmetrical.

In discussing the effect of lesions on the descending spinal reflex activity, it has been assumed that the action of such lesions is to interrupt or not, as the case may be, fibers conveying descending tract impulses, and there is little doubt but that this assumption is substantially correct. However, it must be remembered that postbrachial lesions of the ventrolateral columns affect decerebrate rigidity and stretch reflexes in the brachial field (7). It might be that a hemisection, for example, by virtue of some action analogous to the Schiff-Sherrington phenomenon, affects the number and pattern of descending impulses above the lesion as well as below.

Functional organization of the long spinal reflex mechanism. Figure 18 presents in diagrammatic form a survey of the functional connections established between the descending tracts subserving long spinal reflexes, interneurons and motoneurons of the lumbar enlargement. There is fragmentary evidence in the anatomical literature to show that neurons in the cervical cord which possess descending axons, lie in the depth of the ventral horn. The descending axons, at least in the upper lumbar region, lie in the lateral and ventral columns (Fig. 17). In Fig. 18, then, collaterals of the tract fibers are pictured as entering the two ventral horns from the lateral and ventral columns of the side ipsilateral to the afferent stimulation, and from the ventral column alone on the contralateral side (Fig. 15, 16, 17). The tract collaterals end on interneurons through the ventral horn, some being unilateral, others commissural (Fig. 6, 7, 8, 9). For purposes of description the unilateral interneurons are grouped together as the *nucleus proprius cornu anterioris* (N.Pr.C.A.), regardless of whether the functional grouping has a true anatomical entity as a nucleus or not. Commissural neurons are grouped into the *nucleus cornu-commissuralis anterior*, for which there is considerable functional and anatomical justification.

The first tract impulses reach the lower spinal mechanism at 2.5 to 3 msec. after the brachial plexus shock, and act in a way so as to result in inhibition of certain two-neuron-arc reflexes. After the lapse of another 3 to 5 msec. interneurons within the ventral horn become active, and it is at this time that the first excitatory action on motoneurons has been detected. It is only after an additional 2 to 3 msec. that the motoneurons in turn discharge impulses to the periphery. The time interval that exists between the arrival of excitatory impulses within a given nucleus and the discharge of impulses

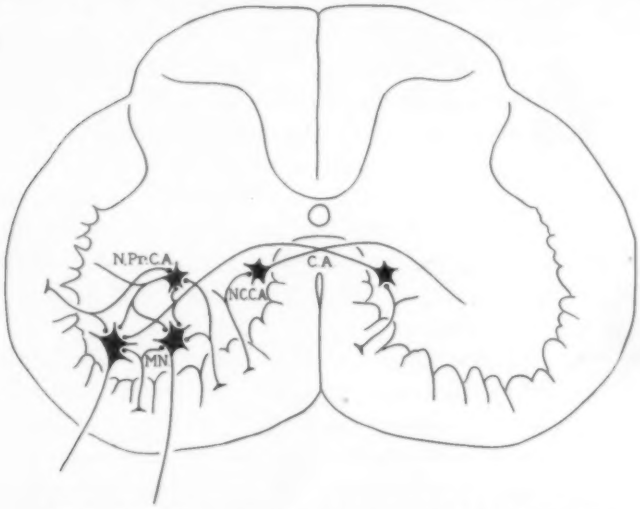

FIG. 18. Diagrammatic representation of the functional organization of the lower spinal mechanism to illustrate the connections established by long spinal reflex action. C.A., anterior commissure; M.N., motoneurons; N.C.C.A., nucleus of the anterior commissure; N.Pr.C.A., internuncial nuclei of the ventral horn.

from that nucleus has been designated as nuclear delay. In some situations, notably at the motoneurons, nuclear delay can be measured, in others it remains conjectural. The same, of course, can be said of synaptic delay. As more knowledge is gained of the dynamics of action within the central nervous system, particularly when nuclei are activated by diffuse discharges (occasioned by simple conduction dispersion or by antecedent nuclear intervention) rather than by highly synchronized volleys, it becomes increasingly clear that nuclear delay is a phenomenon of general significance. Central latency is the sum of central conduction and nuclear delays. All this, of course, does not explain nuclear delay. The simplest assumption is that it is the time necessary for the diffuse influx of impulses to reach an intensity sufficient to secure discharge from a nucleus. This assumption is undoubtedly valid for many nuclei under various conditions of activation, but it may not prove valid for all. It should be noted that the synaptic delay of Sherrington encompasses both the synaptic delay and the nuclear delay of contemporary description.

There is at the present time no clear picture of the anatomical substrate of direct inhibition. Consequently no adequate attempt is made in Fig. 18 to depict inhibitory connections.

Throughout the activity period engendered by brachial plexus stimulation, the dorsal regions of the cord remain silent and play no immediate part in long spinal descending activity. In this connection the contrast between the spinal mechanism as activated by the long spinal reflex system and by the pyramidal system (4, Fig. 14) is striking. The contrast emphasizes the nicety and precision with which various descending systems fractionate the spinal mechanism. At the present time there is little knowledge by which to decide whether the fractionation is predetermined by limitation of anatomical interconnection or whether it is brought about primarily by functional means.

Summary

Some aspects of the transmission of reflex effect from the forelimb to the hindlimb have been examined. Such transmission of necessity employs the descending propriospinal neurons, long and short, as a mediate system.

Transmission of activity arising on one side of the body involves propriospinal tracts of both halves of the spinal cord, as a result of the free yoking together of the brachial mechanisms. Free decussation occurs again in the lumbar cord. Consequently unilaterally evoked activity, by transmission through the propriospinal system, excites motoneurons by strictly unilateral paths, by decussating or crossed paths, and also reaches motoneurons of the same side through doubly decussated paths. The lateral columns are more intimately concerned with strictly unilateral transmission. The ventral columns subserve both unilateral and bilateral transmission. No significant functioning in the present experiments can be attributed to the dorsal column.

The first effect exerted in the lumbar cord by a brachial plexus volley is inhibition of some motoneurons of the ipsilateral side through unilateral pathways. The latency of inhibition allows only for conduction and a single synaptic relay in the cervical cord between primary afferent and propriospinal fibers. Hence long propriospinal fibers are involved and the inhibitory effect is a direct action.

Shortly after the onset of inhibition, interneurons of the lumbar enlargement become active and the motoneurons simultaneously are facilitated. At the height of the facilitation period, which lasts *ca.* 35 msec., motoneurons discharge impulses to the periphery. The long spinal reflex discharge is much more variable than short spinal reflexes, but under favorable conditions may approximate or even exceed the latter in size and synchrony.

In the lumbar enlargement the active neuron pools are confined to the ventral half of the cord. The dorsal horns and most, if not all, of the intermediate region remain inactive. In the ventral horn the most powerful activity is encountered in the *nucleus cornu-commissuralis anterior*, indicating the important role played by crossed mechanisms.

A degree of mutual interference takes place between long spinal reflex activity and short spinal reflex activity pertaining to multineuronal paths. This interference is designated as "mutual inhibition," for the deficit is frequently too great to be accounted for as occlusion. On the contrary, long spinal reflexes reinforce one another.

When nuclei are activated by diffuse discharges rather than synchronized volleys, synaptic delay is no longer the determining factor (along with conduction) in central latency. Nuclear delay, usually having several times the duration of the longest known central synaptic delays, becomes a characteristic feature of transmission and accounts for the greater part of central reflex time.

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INTERSEGMENTAL INHIBITION IN THE SPINAL CORD OF THE FROG

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(Received for publication July 13, 1942)

IT HAS long been known that in frogs exposed to cold a peculiar state of increased reflex excitability develops. (For the older bibliography see [2].) Most of the former investigations have been complicated by cooling of the whole frog by packing it in ice. Stimulation of the skin by cold and the tendency of cooled nerves to tetanic discharge may act to increase reflex excitability. However, Biedermann (2) has demonstrated that the increase of excitability occurs when these sources of error are avoided. He has interpreted the phenomenon as a change in the metabolism of the spinal cord during cooling. Von Baeyer (1) failed to observe any accentuation of the increased excitability of strychninized frogs as a result of cooling; on the contrary the reflex discharge appeared to be diminished. According to some older observations (1), strychnine tetanus may be entirely prevented by cooling.

Recently, Ozorio de Almeida (4–10) and his co-workers have published a series of papers on "epileptiform cramps" in the South American frog, *Leptodactyllus ocellatus*, induced when the isolated spinal cord (not separated from the vertebral column) was suddenly immersed in cold solutions but not when the solution was gradually cooled. Ozorio de Almeida *et al.* have described a "jump reflex" in the spinal frog elicited by allowing the frog to fall from a certain height on the sole of the foot and have shown it is increased by cooling.

Further to elucidate the effect of cold upon the central nervous system, we have used Winterstein's (11) isolated spinal cord preparation so that all interfering factors are avoided.

METHOD

The spinal cord of *Rana esculenta* was entirely isolated from the vertebral canal as far as the cauda equina and left connected by the sciatic nerve with the entire leg or with the lower parts only. The cord was immersed in oxygenated Ringer's solution, contained in a small cylindrical vessel, which was surrounded by a second cylinder of water to keep the Ringer's solution at any desired temperature. By this method, only the spinal cord and nerve roots were exposed to the change in temperature.

The ipsilateral reflex of one leg was elicited by electrical stimulation of the skin of the foot by fine wires which did not hinder movement of the leg. For registration of the reflex movement, the toes were connected by a thread to a straw lever writing on a smoked drum. To avoid fatigue the time interval between stimuli was rather long. In most experiments we employed a device producing a faradic current at a frequency of 15 per sec. and a duration of 1.5–2 sec. every 3 min.

RESULTS AND INTERPRETATION

When the isolated spinal cord preparation was cooled down to temperatures of 12°–2°C. (in most experiments 8°–5°C.) there always occurred as an end result a *decrease* in reflex action, usually to the point of complete abolition

of excitability. In most experiments excitability was restored after raising the temperature to previous levels.

At the beginning of cooling the behavior of the various preparations was quite irregular. In more than half of the 38 experiments, a considerable initial increase of reflex response was observed before the onset of depression. The threshold for producing a minimal response, however, remained unaltered. This behavior was not influenced by changing the surrounding medium of the preparation (oxygenated-Ringer's solution or an atmosphere of pure oxygen). In order to decide whether the irregularity of results was due to differential cooling of various parts of the spinal cord, regional cooling by means

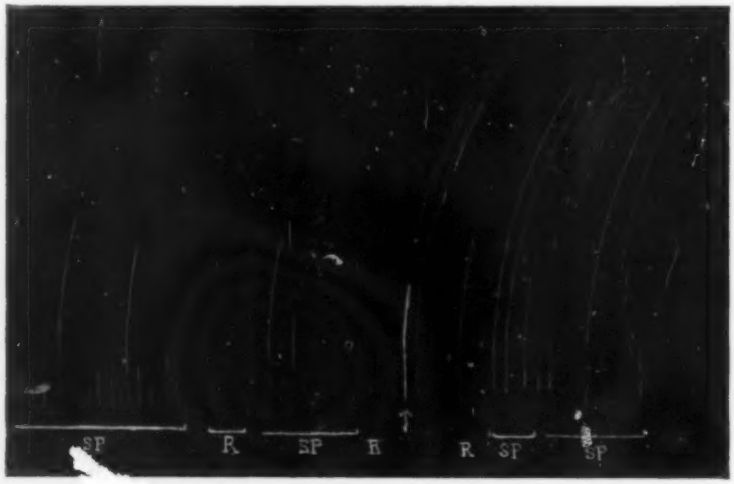

FIG. 1. Effect of cutting (↑) on the reflex (R) end the spontaneous (SP) movements.

of thermodes of cervical, dorsal, and lumbar regions was attempted. However no distinct differences could be established.

In spite of this negative finding several considerations suggested that the cause of the initial increase of reflex activity lies in the *removal by cold of some intraspinal inhibition*. Therefore the effect of section of the isolated spinal cord at various levels on the reflex activity of the segments below the section was investigated. An enormous increase in the magnitude of reflexes occurred in 17 of 19 experiments when the isolated spinal cord was sectioned at the cervical or upper dorsal level. This increase, however, was especially marked after section just above the lumbar enlargement. The threshold stimulus here too remained unchanged, which suggests that removal of an inhibitory influence normally exerted by the upper spinal segments on the lumbar segments is responsible for the phenomenon.

Cooling after sectioning the spinal cord at the lower thoracic level did not produce the initial increase in reflex excitability often observed when the whole isolated cord was exposed to low temperatures. The most convincing results on this point were obtained when the effect of cooling before and after

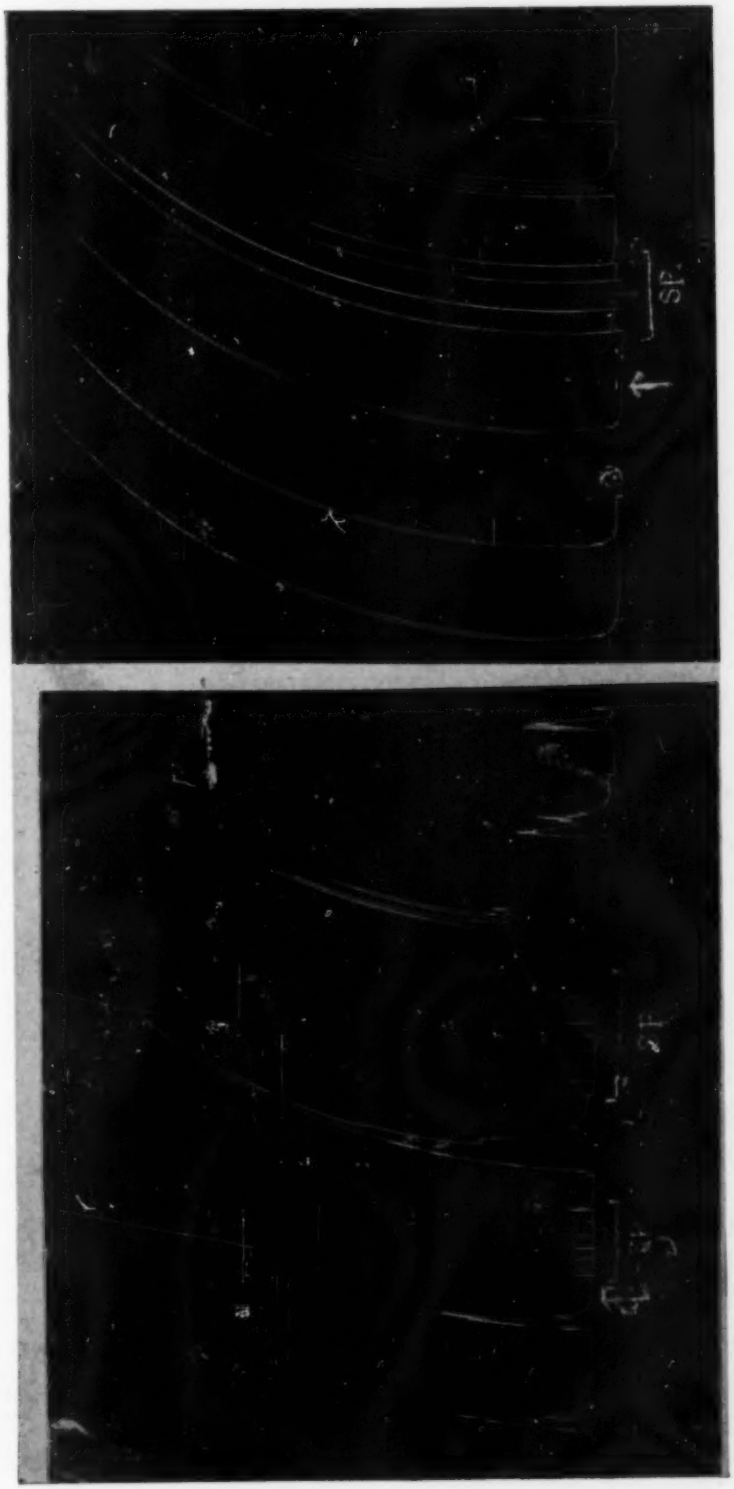

FIG. 2. Effect of cooling (↑) before (A) and after (B) section of the spinal cord on the spontaneous (SP) and the reflex (all not marked) movements.

cutting the spinal cord at the dorso-lumbar level was investigated. In 5 experiments an initial increase in reflex response in cooled preparations was observed before section of the cord. However, subsequent to the section an immediate diminution of reflexes resulted from cooling. Figure 2 demonstrates these effects and the enormous increase of reflexes following section is also to be seen.

These results suggest an interpretation of the behavior of the intact isolated cord to cooling. The initial increase of reflex excitability when the intact cord is cooled may be explained in the same way as the effect of sectioning the spinal cord. The effect of cooling may always be to reduce reflex activity; but cephalic segments exercising an inhibitory influence over lumbar reflexes may in some cases be the first affected by cold and thus their inhibitory action may be abolished. If this were true there would result an increase of reflex action which would persist until the lumbar segments in turn were inactivated by cold. Thus after the removal of the inhibitory mechanisms by cord sections only the depressing effect of cooling may be observed.

In addition to changes in induced reflexes from section and cooling of the spinal cord there are similar alterations in spontaneous movements. After cord section spontaneous movements also are increased in magnitude and frequency in some experiments. Cooling of the spinal cord acts similarly. In some preparations such movements did not occur at all until the cord was cooled. Spontaneous movements were observed not only in intact cord preparations but also after isolation of the lumbo-sacral segments (see Fig. 2). Analysis proves them to be of reflex origin. They disappear when the posterior spinal roots on both sides are sectioned. Their number may be greatly diminished or they may cease entirely after removal of the skin. The fact that these reflexes are augmented or appear only after cutting and cooling of the spinal cord proves that they are also subject to inhibition from cephalic cord levels. But since spontaneous and induced reflexes are independently and sometimes oppositely changed by cooling, the central mechanisms of the two may differ.

Conclusions

The effect of cooling and of sectioning the spinal cord of the frog when separated from the brain and removed from the body has been investigated.

1. Prolonged cooling of the spinal cord diminishes or abolishes ipsilateral reflex responses to electrical stimulation of the skin of the foot.

2. Diminution of reflex activity is often preceded by an increase.

3. Section of the spinal cord, especially just above the lumbar enlargement, regularly produces a marked increase of reflex response. The threshold strength of current remains unchanged in all three cases.

4. Cooling subsequent to low dorsal section always depresses reflex activity.

5. Spontaneous movements, proved to be of reflex origin, behave similarly to induced reflexes.

6. These observations are interpreted as follows: intersegmental inhibitory mechanisms involving the cervico-dorsal segments of the cord are affected by cold earlier than segmental reflex arcs of the lumbar region. Removal of such inhibition by cooling or by cord section increases the magnitude of reflex responses.

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OSCILLOGRAPHIC STUDIES ON THE SPINAL TRACT OF THE FIFTH CRANIAL NERVE*

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(Received for publication July 21, 1942)

THE DEVELOPMENT of trigeminal tractotomy by Sjöqvist (40) for alleviation of facial pain brought about renewed interest in the anatomy and physiology of the spinal tract of the fifth cranial nerve. The tract has been studied clinically and experimentally by many workers, but there is disagreement concerning its role in mediation of sensations other than pain and temperature. Although the evidence is good that touch is mediated both by the chief sensory nucleus and by the spinal nucleus, there are many who still maintain that the spinal nucleus receives impulses elicited only by pain and temperature. Confusion exists also in regard to the relative location in the brain stem of fibers from the peripheral divisions of the trigeminal nerve and the caudal limits of their course.

Because of the preciseness of localization possible with oscillographic recordings within the brain stem, this technic was employed in studying the spinal tract. It was possible to record within the root the location of activity arising in each of the three divisions and to trace the activity to its caudal reaches.

MATERIALS AND METHODS

Twenty cats anesthetized with sodium pentobarbital were used in these experiments. A unipolar lead was oriented within the brain stem with the Horsley-Clarke instrument and electrical activity was recorded with a cathode ray oscillograph and loud speaker. The Horsley-Clarke instrument itself served as the reference electrode. Tactile responses were set up by stroking the skin and hair of the head with a camel's hair brush or blunt glass rod. Synchronous volleys were evoked by electrical stimuli from a thyratron stimulator. The frontal and maxillary nerves were suspended by hook electrodes and a shielded electrode was placed on the lingual nerve. Thus, branches of each of the three divisions of the trigeminal nerve were isolated for study. Impulses elicited peripherally were then traced into the brain stem in order to determine the central distribution of the primary neurones. Photographic records of the cathode ray screen during electrical stimulation and the appearance and sound of activity from tactile stimulation were then correlated with electrode positions as revealed by microscopic sections. The region under consideration was sectioned serially at 40μ, and every third section was stained by the Weil technic.

RESULTS

Upon tactile stimulation of the areas of distribution of the trigeminal nerve, electrical activity in the descending tract of the nerve resembles that characteristic of the activity in a peripheral nerve when its sensory field is stimulated. Tactile impulses originating in the ophthalmic division of the

* A preliminary report of these experiments was given before the American Association of Anatomists, New York, April, 1942.

This study was supported in part by a grant for equipment from the American Association for the Advancement of Science.

fifth cranial nerve pass into the ventral part of the spinal tract and may be traced caudally as far as the caudal third of the first cervical segment. From the mandibular division, similar impulses travel in the dorsal portion of the spinal tract as far as the junction of the medulla and the spinal cord. Potentials elicited by touch over the maxillary distribution were recorded in an intermediate position in the descending tract and were traced to the upper third of the first cervical segment. The fibers from each of the three divisions remain relatively discrete in the spinal tract. As the recording needle was lowered into the tract by 0.5 mm. intervals, tactile impulses appeared more or less discretely, first from the mandibular area, then from the maxillary,

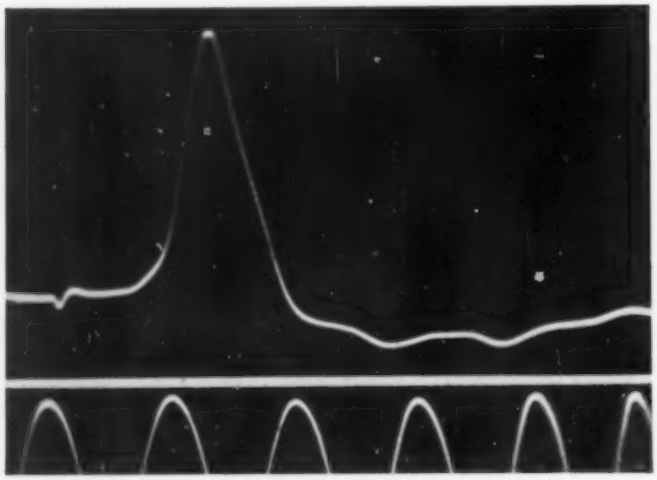

FIG. 1. Photograph of single sweep of cathode ray oscillograph; stimulation of peripheral branch of the trigeminal nerve (infra-orbital); unipolar recording from the spinal tract of the trigeminal nerve; time in msec. given by 1000-cycle wave. Positive potentials are recorded as upward deflections. Further details are given in the text.

and finally from the ophthalmic region. It is evident that in the cat large numbers of tactile fibers pass into the spinal tract of the trigeminal nerve. Tactile impulses may be traced almost as far caudally as a volley set up by a single electrical stimulus to a large peripheral branch of the trigeminal nerve, but, due to the fact that fewer fibers are active at any one time during tactile stimulation, there was uncertainty in delineating the extreme limits reached by those fibers. With the needle in the nucleus of the spinal tract, tactile impulses were recorded in the same dorso-ventral distribution as in the tract itself, although the intensity was less. The higher activity in the tract is due to the fact that at any given position of the needle there are not only fibers termininating medially in the nucleus of the same region but also fibers that continue caudally.

Following a single electrical stimulus to a branch of the fifth nerve, potential waves could be recorded in the spinal tract and nucleus. The action potential varied with the relation of the electrode tip to the spinal tract and its nucleus, and with conduction distance within the brain stem, but in general was made up of two waves each of which had one or more peaks. The first wave began 0.6 to 1.2 msec. after the stimulus, the time depending upon

the conducting distance, and was completed within 2 msec. In many cases, this wave appeared as a discrete spike, but frequently a second peak was recorded and was sometimes higher than the first. Figure 1 is a record from ex-

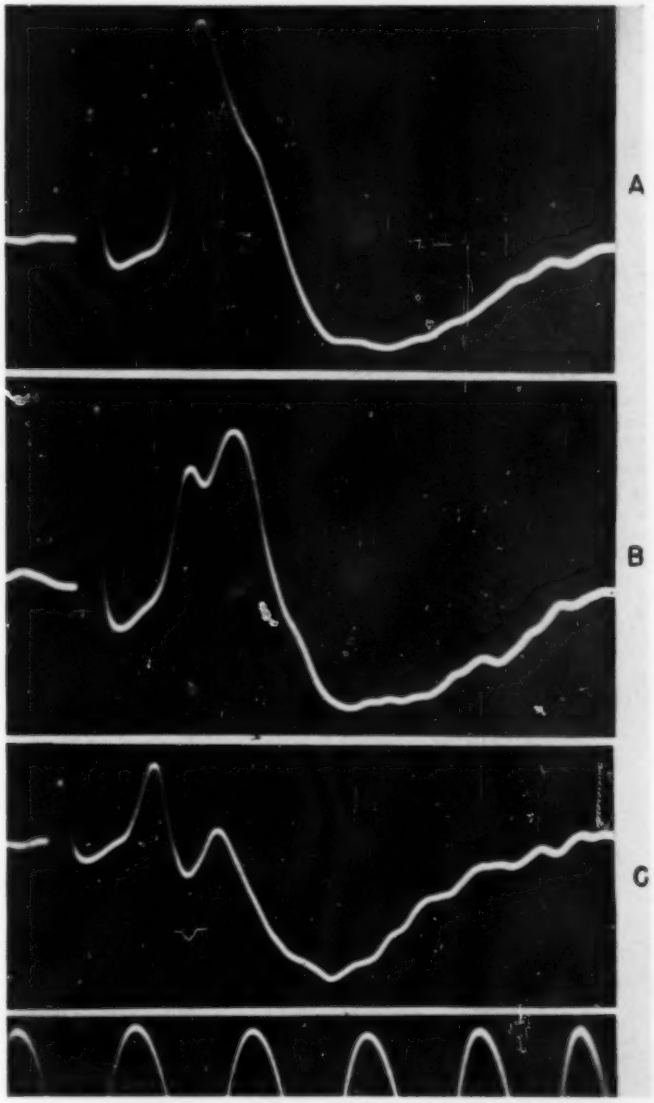

FIG. 2. Lingual nerve stimulated; other details as for Fig. 1.

periment 17 with the needle 13 mm. caudal to the entrance of the root. The action potential began at 0.65 msec., reached a peak at 1.3 msec., and ended at 2.0 msec. Figure 2A from experiment 18, with the recording electrode 3 mm. caudal to the entrance of the root, shows a slight break on the descending limb suggestive of an additional component. Figure 2C is a record ex-

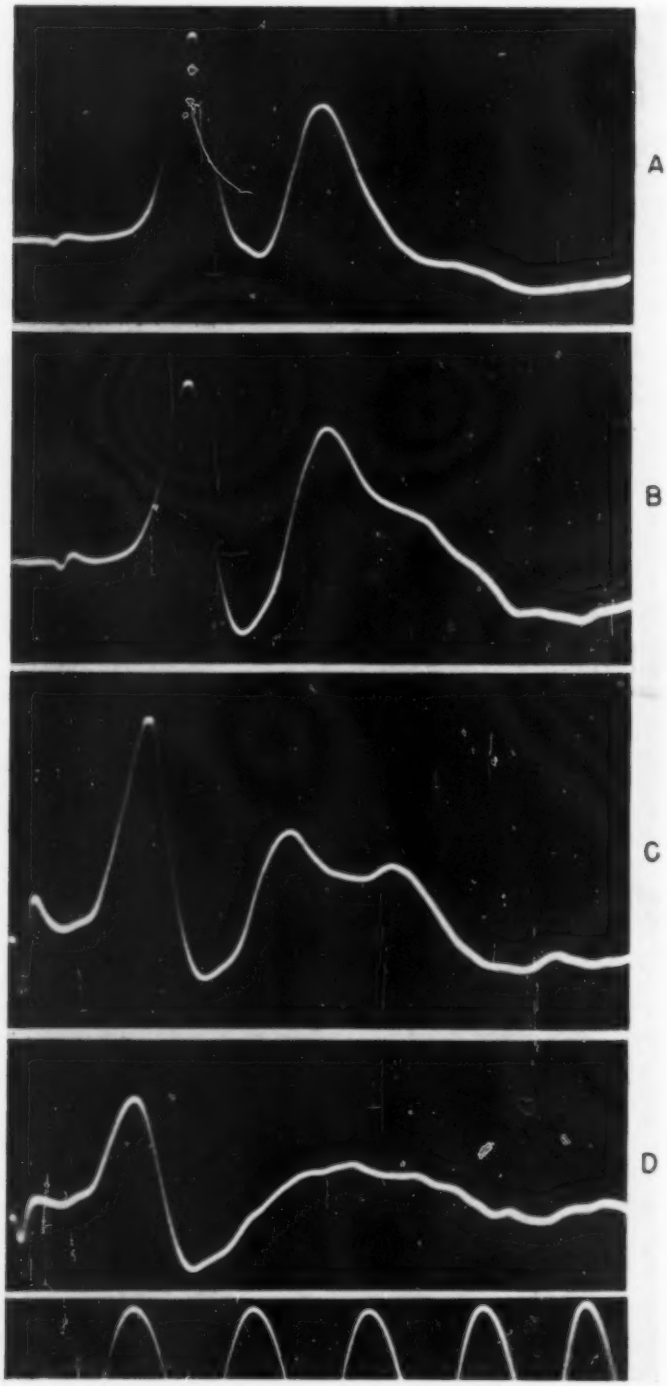

FIG. 3. Details as for Fig. 1.

hibiting two peaks and was taken one mm. dorsal and one mm. medial to that of Fig. 2A. The time course of the first peak was 0.65 msec. to the foot of the spike, 0.9 to the crest, and 1.2 msec. to the end; the time course of the second peak was 1.2 msec. to beginning, 1.45 to crest, and 2.0 to the end. Figure 2B was recorded slightly dorsal to Fig. 2A and had a second peak higher than the first. The peaks were at 0.95 and 1.3 msec., and there was a break on the descending limb at 1.8 msec.

The variation in time from stimulus artifact to the first break in the base

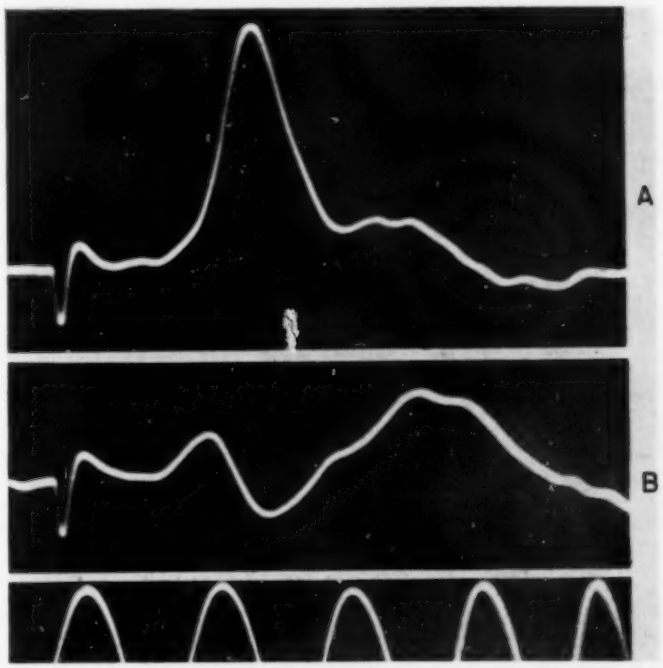

FIG. 4. Frontal nerve stimulated; other details as for Fig. 1.

line varied with conduction distance. The difficulty of measuring conduction distance along the course of the trigeminal nerve makes calculations of conduction rates, at best, close approximations. In seven determinations the average time for beginning of the wave at the entrance of the trigeminal fibers into the pons was 0.67 msec., whereas in eleven determinations the average time at the level of the upper cervical cord was 1.18 msec. The conduction distance between these points was about 25 mm., so that the fastest fibers conducted almost 50 m. per sec. within the brain stem.

The second wave of the action potential began 1.8 to 2.2 msec. after the stimulus. The magnitude and the time course of this part of the action potential were more variable and susceptible to change than was that of the first wave. At any one point, this second wave was constant for given experimental conditions but could be varied considerably or even abolished by

increased frequency of stimulation or oxygen deprivation. This wave varied, just as did the first, in the number of potential peaks. Figure 3A, B, and C are records from experiment 17 showing the changes in form of the second wave with changes in electrode position. As many as four peaks have been seen on the second wave, but with the larger number of peaks the intervals between crests tend to become shorter. At other times a low broad second wave, lasting to 4 or 5 msec. after the stimulus, was evident as in Fig. 3D.

The second wave, that part of the action potential beginning 2 msec. after the stimulus, is very likely due to activity in second order neurones. The wave was small when the recording needle was one millimeter or so dorsal or

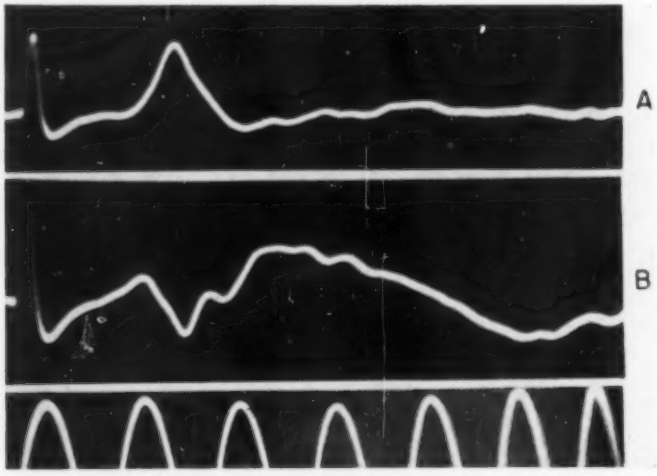

FIG. 5. Frontal nerve stimulated; other details as for Fig. 1.

lateral to the nucleus of the spinal tract. As the needle is moved ventrally or medially, the second wave becomes higher and the first wave smaller. Since the spinal tract overlies the spinal nucleus dorsally as well as laterally, the effect of moving the electrode 2 mm. ventrally from the tract into the nucleus was shown in experiment 16, Fig. 4A and B. The peak of the first wave was inverted and the second wave became larger. Figure 5A from experiment 15, with the needle in the spinal tract, had a small first wave and a negligible second wave, but, as is shown in Fig. 5B, upon moving the electrode one mm. medially, the first wave was inverted and a second wave appeared. Inversion of the first wave does not occur necessarily but depends entirely upon recording conditions (see 26 and 2, for discussions of the relation of the wave form to electrode position in a volume conductor). When the frequency of stimulation was increased from one in 2 sec. to 4 or 5 per sec., a decrease occurred in the second wave, especially in its latter portions, and at frequencies of 40 to 50 per sec. the wave practically was abolished. That oxygen deprivation depressed the second wave is shown by Fig. 6. Respiration ceased between records 6A and 6B, and artificial respiration was employed between

records 6B and 6C. The second wave disappeared within a few seconds after cessation of respiration whereas the first wave was still present several minutes later. In records 6A and 6C, the peak of the first wave was at 1.15 msec., but in record 6B continued to increase until 1.35 msec. The location from which the second wave is best recorded, the decrease in magnitude and duration with slight increases in frequency of stimulation, the susceptibility to oxygen deprivation, and the fact that the crest of the second wave fell 0.5 to 1.5 msec. after the crest of the first are all consistent with the belief that the second wave is produced by second order neurones. At times, a series of 3 or 4 crests occurred on the second wave with an interval of 0.6 msec. from

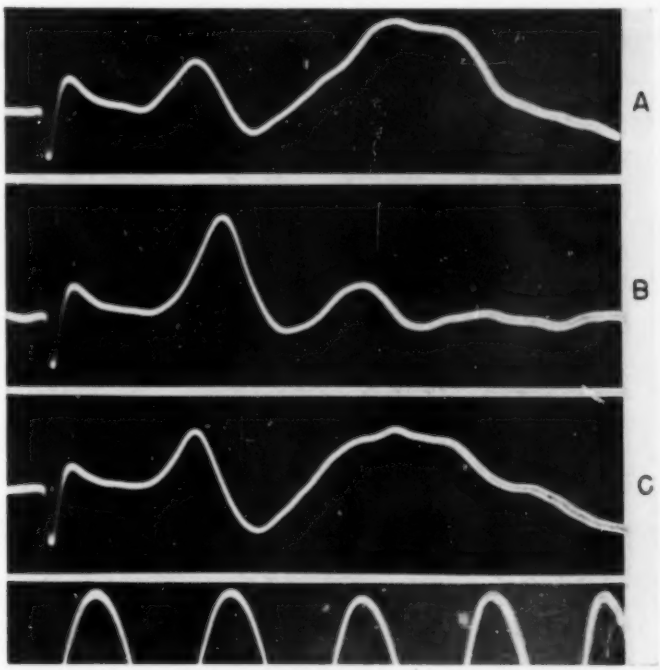

FIG. 6. Frontal nerve stimulated; other details as for Fig. 1.

one crest to the next. Renshaw (33) demonstrated that a single synapse may have a delay of 0.5 to 1.0 msec. but that delays appreciably longer than 1.0 msec. imply more than one synapse. It would be preferable, of course, to measure beginning of activity for each wave rather than the crest in each case but for many of the waves this is not possible. From the beginning of the first wave to the beginning of the second, the range of the delay was 0.8 to 1.1 msec. which is within the limits of a single synapse.

The potential waves elicited electrically were easier to trace than those from tactile stimulation but, even so, could not be followed significantly farther caudally. Figure 7 from experiment 16 illustrates the delineation of the caudal extent of the root. The frontal nerve was stimulated and records

were taken successively: Fig. 7A, with the needle in the dorso-lateral fasciculus of the caudal third of the first cervical segment, shows practically no activity; in 7B, one mm. rostrally, a small but definite first wave appears and shows that the caudal fibers of the spinal tract now are being contacted; and in 7C, one mm. farther rostrally, the presence of a large first wave shows that the recording electrode is now in contact with still more fibers. Similar observations were made for the caudal extent of the maxillary and mandibular fibers but at correspondingly higher levels.

Figures 8 to 13 are projection drawings from the brain stems of cats 6 and 10 and show diagrammatically the cross sectional relationships of the trigeminal divisions in the spinal root at different levels. Figure 14 is a dia-

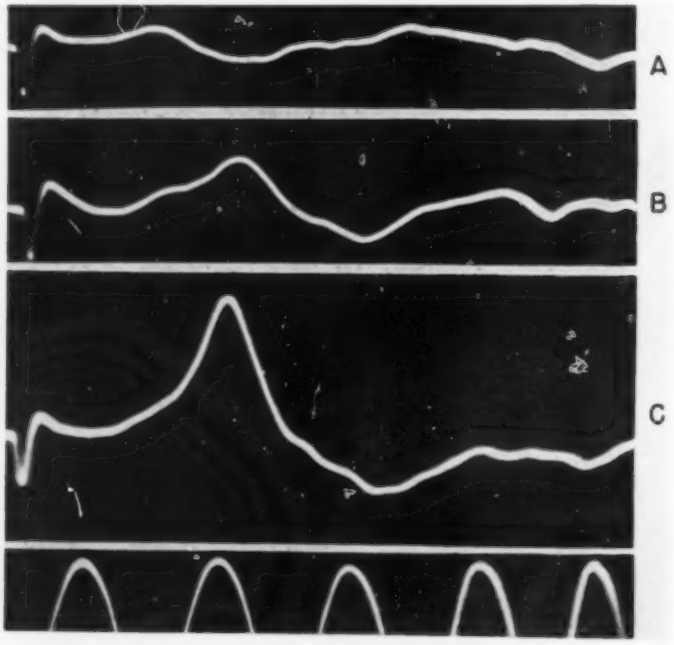

FIG. 7. Frontal nerve stimulated; other details as for Fig. 1.

gram of the dorsal aspect of the pons, medulla, and cervical cord showing the course and caudal extent of three divisions.

DISCUSSION

Section of the spinal tract of the trigeminal nerve was carried out experimentally many years before being introduced as a surgical procedure for alleviation of facial pain. Laborde (25), in 1877, cut the tract in rabbits and dogs in order to compare the results with those obtained by earlier workers who sectioned the divisions of V or its sensory root. Observations were made chiefly on the eye, and loss of sensation and trophic changes were described. Sherrington (39), Ferrier (9), Biedl (1), and Van Gehuchten (12) were among others who experimentally sectioned the tract.

Ranson (32), in a paper read before the New York Neurological Society, presented his views on the fibers types in the spinal tract. In the discussion of Ranson's paper, Kuntz (p. 1140) and Elsberg (p. 1142) each suggested the possibility of cutting the tract for neuralgic pain. The main paper and dis-

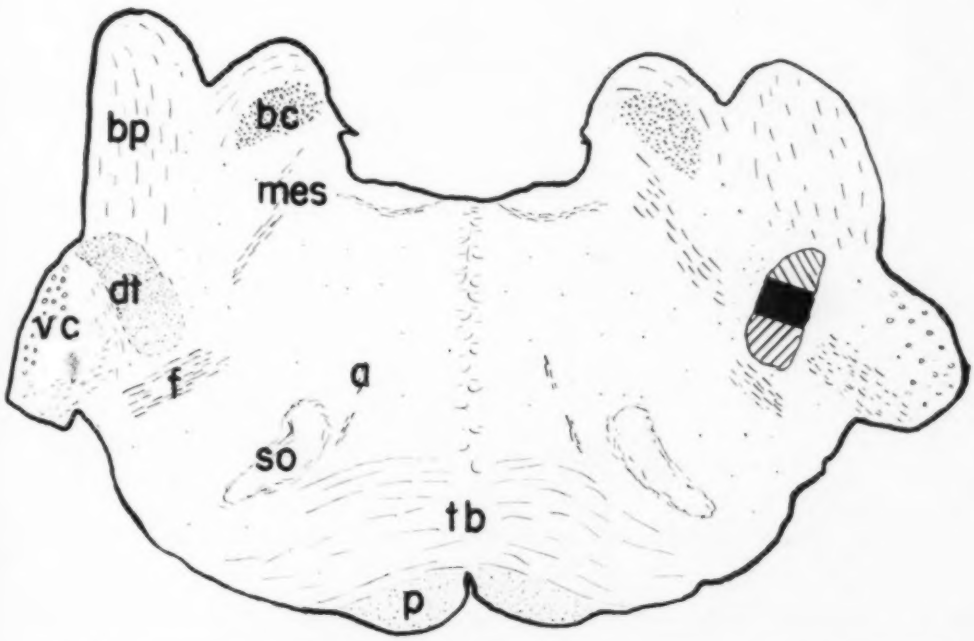

FIG. 8. Projection drawing of cross section of brain stem of cat 6. The positions of the fibers of the three divisions are shown diagrammatically on the right side; the dorsal cross-hatched area represents the mandibular fibers, the black area represents the maxillary fibers and the ventral cross-hatched area the ophthalmic fibers. This section is at a level just caudal to the entrance of the fibers into the pons. Abbreviations for all figures: a abducens fibers; AA area acoustica; AC ala cinera; AM anterior medullary velum; anc accessory cuneate nucleus; BC brachium conjunctivum; bp brachium pontis; Cl first cervical segment; CN cuneate tubercle; CP cerebellar peduncle; dp decussation of the pyramid; drcl dorsal root of the first cervical segment; dt descending or spinal tract of trigeminal nerve; dvr descending vestibular root; f facial fibers; fc fasciculus cuneatus; fg fasciculus gracilis; gf genu of facial nerve; GN gracile tubercle; HT hypoglossal trigone; hy hypoglossal fibers; IC inferior colliculus; io inferior olive; mes mesencephalic root of trigeminal nerve; nc nucleus cuneatus; ndt nucleus of the descending tract; ng nucleus gracilis; nhy hypoglossal nucleus; p pyramid; rb restiform body; so superior olive; svn superior vestibular nucleus; t trigeminal nerve; tb trapezoid body; TC tuberculum cinereum; ts tractus solitarius; vcn ventral cochlear nucleus; V1 ophthalmic division of the trigeminal nerve; V2 maxillary division of the trigeminal nerve; V3 mandibular division of the trigeminal nerve; VII facial nerve; VIII acoustic nerve.

cussion centered on the fact that here was an anatomical separation of functional fiber types and the possibility that pain could be abolished without loss of touch. Sjöqvist (39a) was unaware of the above discussion at the time he decided that section of the spinal tract was a feasible and worthwhile operation for relief of certain types of facial pain. In a monograph later the same year, Sjöqvist (40) referred to Ranson's paper and discussed

in detail the rationale of the operation which now bears Sjöqvist's name. Additional cases in which trigeminal tractotomy has been utilized clinically

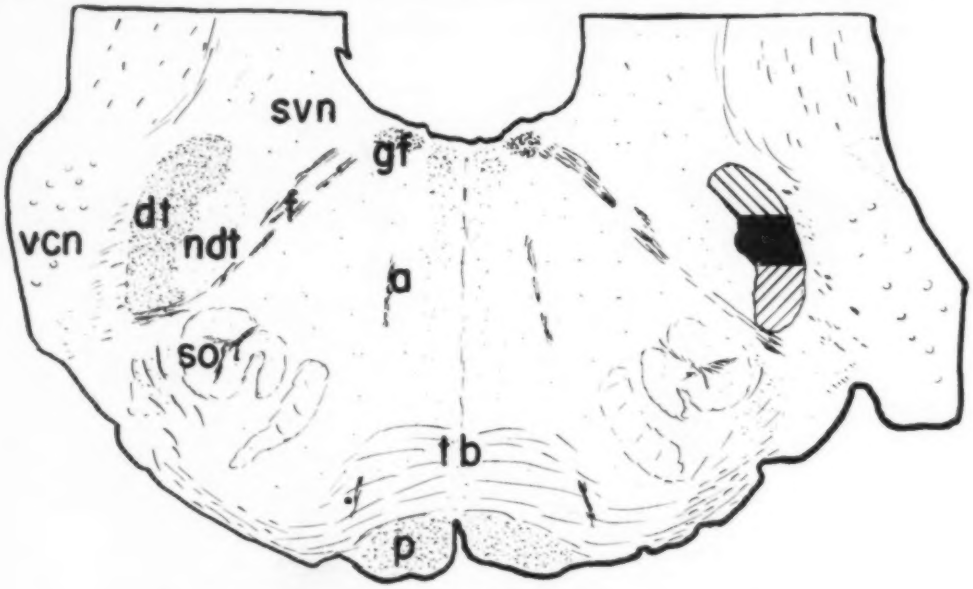

FIG. 9. Drawing of a section of the brain stem of cat 6 at the level of the facial nerve.

have been described extensively (15, 16, 17, 18, 22, 23, 29, 34, 35, 36, 41, 42). Using a similar approach, Schwartz and O'Leary (37) sectioned the spino-

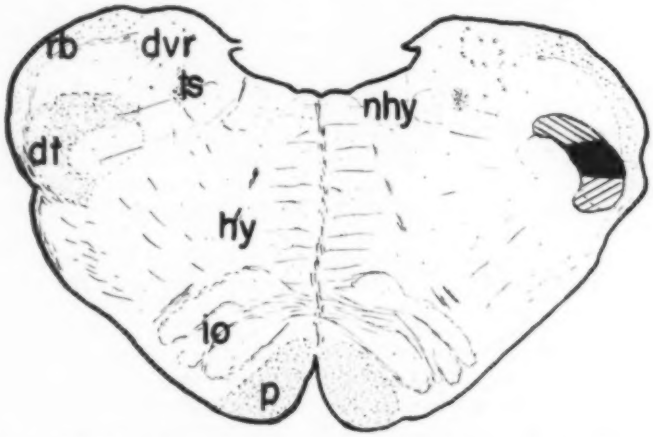

FIG. 10. Drawing of a section of the brain stem of cat 6 at the level of the hypoglossal nerve.

thalamic tract and part of the spinal trigeminal tract for intractable pain.

The strongest point in favor of tractotomy over root section by the Spiller-Frazier or Dandy methods is that touch grossly is preserved and

therefore there is less likelihood of subsequent trauma to the eye or other facial structures. Many investigators (5, 14, 21, 22, 42, 47, 48, 49, 50, 60), claim that the spinal nucleus receives only fibers of pain and temperature or state that there is no loss of tactile sensibility from lesions of the tract. Some

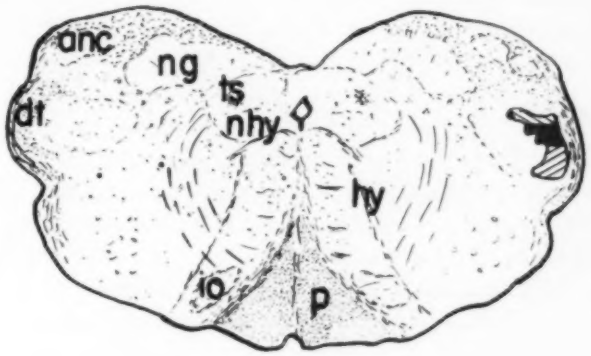

FIG. 11. Drawing of a section of the brain stem of cat 6 just caudal to the obex.

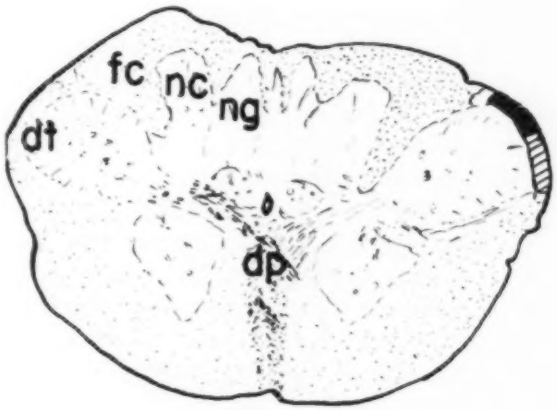

FIG. 12. Drawing of a section of the brain stem of cat 6 at the junction of the medulla and cord.

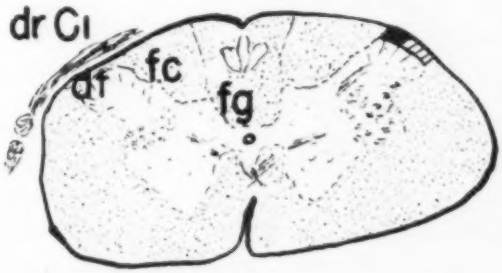

FIG. 13. Drawing of a section of the brain stem of cat 10 at the level of the rostral part of the first cervical segment.

workers very emphatically insist that touch impulses do not go to the spinal nucleus. As an example, Spiller (47), states, "In several papers, the first of which appeared in 1908, I showed that the spinal root of the trigeminal nerve contains pain and temperature fibers and does not contain fibers of tactile sensation."

However, careful clinical studies of patients subjected to trigeminal

tractotomy reveal that the spinal tract and nucleus also may mediate touch. Van Valkenburg (55) believed that touch is received by the spinal nucleus. Anatomically, the bifurcation of many trigeminal fibers as they enter the pons permits impulses to go both to the chief sensory nucleus and to the spinal nucleus. Windle (58) discussed this problem of the bifurcating fibers and decided that touch impulses travel both routes. Ranson (32) also pointed out the clinical implications of this anatomical arrangement. In the discussion (p. 1144) he stated, "I believe that these descending branches of the coarse fibers carry tactile impulses, and I believe that touch is mediated not only by the main sensory but also by the spinal nucleus, and therefore that when there is a lesion of the spinal fifth tract there may be some dulling of tactile sensibility. I should say that pain and temperature are mediated exclusively through the spinal fifth nucleus and touch through both the main sensory and the spinal fifth nuclei." This view has been substantiated fully by careful studies with von Frey hairs of patients with trigeminal tractotomy. Such studies have been carried out by Walker (56), Groff and his collaborators (15, 18), and Grant and Weinberger (16, 17). Sjöqvist (40), Rowbotham (34), and Olivecrona (29) also mention that, following tractotomy, the sensation of touch on the operated side is not normal. It thus seems that much of the confusion in the literature has resulted from the fact that many workers have tested for preservation of touch by stroking the face with cotton-wool but have not carried out the crucial tests with graded hairs as have the recent workers mentioned above. Such tests invariably reveal a measurable, though not necessarily disturbing, loss of tactile sensibility. Even though touch fibers do pass into the spinal tract it is likely that most of them are collaterals from fibers passing to the chief sensory nucleus. Therefore the statement

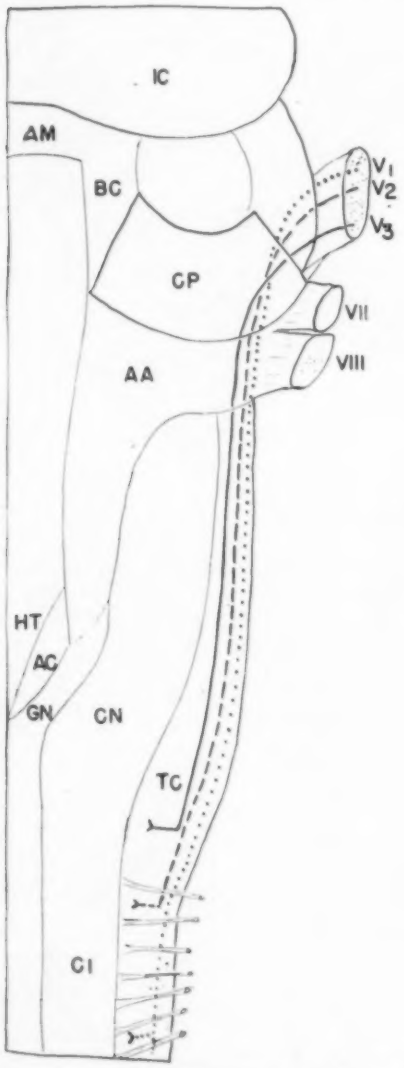

FIG. 14. A diagram of the dorsal surface of the pons, medulla, and upper cervical cord showing the course and level of termination of the fibers of the three trigeminal divisions.

of Grant and Weinberger (16) that, "Touch sensation for all practical purposes is preserved, and the patients are hardly aware that their faces have been made analgetic," undoubtedly is correct.

The experiments reported here show that in the cat a large number of tactile impulses can be traced into the spinal tract as far as the lower medulla and first cervical segment. In the monkey, Sjöqvist (40) found medium and large fibers in the spinal tract, presumably for mediation of touch, which disappeared before reaching the inferior olive. Gerard (14) and Windle (59) described similar medium to large sized fibers in the spinal tract of the cat.

There has been rather general agreement on the dorso-ventral arrangement of the trigeminal fibers within the spinal tract. Although many workers had sectioned successfully the sensory root in experimental animals and traced the central degeneration, Bregman (4) was the first to follow the degeneration centrally after partial section of the root. Upon cutting the sensory root in rabbits in such a manner that the corneal reflex was abolished, degeneration was found in the medial part of the sensory root and in the ventral part of the spinal tract. Sections of the root preserving the corneal reflex left the ventral part of the tract intact and degeneration was confined to the lateral part of the root and dorsal part of the tract. These findings were supported by the work of Wallenberg (57), cat and rabbit, by Bochenek (3), rabbit, Spiller and Frazier (46), dog, (11, for discussion) Van Valkenburg (54), various animals, and by Davis and Haven (7), cat. Without exception, these earlier workers found the fibers from the ophthalmic division in the ventral part of the spinal tract, the maxillary fibers in an intermediate position, and the mandibular fibers placed dorsally.

Clinical evidence supports the above experimental findings (15). Olivecrona (29), in describing his procedure, states, "If the incision has been placed correctly the pain felt at the moment of incision is referred to the entire trigeminal level. If pain is felt only in the first division, the incision should be extended a little more in the dorsal direction, since the fibers belonging to the third division lie in the dorsal part of the tract. Conversely, if pain is felt only in the third division, extension in the ventral direction is indicated." The only recent clinical report suggesting any arrangement other than that indicated is by Schwartz and O'Leary (37), who described loss of tactile sensibility over the mandibular distribution following damage to the ventrolateral part of the spinal tract in a single human case.

In the experiments here reported, there is direct physiological confirmation that in the spinal tract mandibular fibers are placed dorsally and ophthalmic fibers ventrally. Impulses from the ophthalmic distribution could be traced into the ventral part of the tract. The results indicate that there is little intermixture of the fibers of the three divisions within the spinal root. Pitts (30) used the spinal tract of the trigeminal to test localization of bipolar stimulating electrodes, and his Fig. 3 shows that, upon recording from the frontal nerve and stimulating centrally, the best response was obtained with the needle in the ventral third of the tract. Practically no response was

obtained from the frontal nerve with stimulation of the dorsal third of the tract. In a study of the afferent trigeminal pathways, McKinley and Magoun (27) also stated that the three divisions are laminated in inverse dorsoventral order.

The caudal extent of each of the divisions has been a source of much disagreement, although uniformity of opinion has existed regarding the caudal terminations of the tract as a whole. The present experiments indicate that there are enough fibers from the ophthalmic division in the tract at the level of the caudal third of the first cervical segment to produce measurable potentials. Levels reported by others as the caudal extent of the spinal tract are: for the cat, Biedl (1), C2, Wallenberg (57), C1, Kljatschkin (24), C2, and Davis and Haven (7), C1; for the rabbit, Bregman (4), upper cervical cord, Laborde (25), the point of the calamus, Van Gehuchten (12, 13), lower C2, and Bochenek (3), at least C1; for the monkey, Tooth (53), upper C2, Sherrington (39), midway between C2 and C3, Ferrier and Turner (10), C2, and Ferrier (9), C2; for the guinea pig, Soukhanoff (44), upper cervical cord; for man, Obersteiner (28), C2, Poniatowsky (31), C2, Hun (21), upper C1, von Sölder (43), C2, Dejerine (8), C4, and Sjöqvist (39a), at least to C1. The fact that several species do not have a first cervical sensory root and that the sensory field of the second cervical nerve is contiguous with the trigeminal has led many workers to conclude that centrally the incoming sensory trigeminal neurones should be expected to reach the level of the incoming second cervical dorsal root. From the levels given above, it is evident that this is true. The description by Davis and Haven is typical of the degeneration studies in that, as the tract was followed caudally after section of the ophthalmic fibers, degeneration included an increasingly greater part of the ventral portion of the tract until, in the lowest sections, almost the entire tract was involved. The decrease in mandibular and relative increase in ophthalmic fibers caudally is indicated in Fig. 11, 12, and 13.

The results obtained here concerning the differential endings of the three divisions are consistent with the view most widely accepted at the present time. The levels were the lower part of C1 for the ophthalmic, the middle of C1 for the maxillary, and the junction of the medulla and C1 for the mandibular division. The conclusion that the ophthalmic division reaches farthest caudally was reached by many investigators (4, 5, 7, 29, 45, 50, 51, 54, 57). The alternative view that the distribution of fibers in the spinal tract is reflected peripherally as concentric rings converging on the bucco-nasal openings has been presented in varying forms by others (8, 43, 60). This view, which has been rejected by the majority of the workers on this subject, is discussed in full by Smyth (42) and Stopford (49). Since the spinal nucleus of the fifth nerve extends rostrally almost to the level of entrance of the trigeminal fibers and, since collaterals from the tract turn into the nucleus in abundance in its rostral part, it would be logical to suppose that section of the tract for relief of facial pain should be carried out as high as possible. The highest level that was considered safe by Sjöqvist (40) was the level of the

lowest vagal rootlet, at about the junction of the middle and caudal thirds of the inferior olive. Section at this level resulted in analgesia over all divisions peripherally but occasionally damaged the vagus nerve and restiform body. Grant and Weinberger (16), in later operations, decided that it was unnecessary to make the section so high, having found analgesia quite complete by making the incision 4 to 5 mm. below the obex and 2 mm. below the olive. This is said to be 12 to 14 mm. caudal to the point recommended by Sjöqvist. In one case, a section 8 mm. below the obex gave total and lasting anesthesia in all three divisions. Olivecrona (29) also decided, on the basis of several operations, that the Sjöqvist level was too far rostral and therefore sectioned at the level of the caudal pole of the olive, 2 to 3 mm. caudal to the level originally recommended. Olivecrona found that analgesia was just as complete when the trigeminal tract was cut at the lower end of the fourth ventricle as when it was divided 3 to 5 mm. higher. He concluded that comparatively few fibers leave the spinal nucleus to enter the secondary trigeminal pathway above the level of the lower end of the fourth ventricle. The many morphological descriptions of the spinal tract agree that the cross sectional area decreases slowly to the level of the exit of the hypoglossal nerve, decreases at a more rapid rate to the level of the decussation of the pyramids, and then quite rapidly as the cervical cord is reached. Sjöqvist's figures for the monkey show that the area at the level of the superior olive is 2 sq. mm., at the level of the entrance of the acoustic nerve, 2 sq. mm., at the caudal end of the inferior olive, 1.5 sq. mm., and at the pyramidal decussation, 1.5 sq. mm. Although it is not surprising that a high percentage of fibers in the spinal tract reaches as far as the lower medulla, it is interesting that the numerous collaterals given off at higher levels are not better able to carry on function in the cases operated for facial pain. In the preliminary report of McKinley and Magoun (27) it is stated that the axons of the second order pass diffusely from the main sensory and the spinal fifth nuclei, a statement made on the basis of oscillographic recording and one which would be expected from the anatomical arrangement.

In a review of all cases of Gasserian ganglionectomy up to 1896, Tiffany (52) stated that, "the history of operative measures for the relief of facial neuralgia is a history of operations on nerves at first peripheral, advancing slowly centrally." Since that time, the operative approach has progressed to the root section carried out by Spiller and Frazier (46), and to trigeminal tractotomy carried out by Sjöqvist. Serra and Neri (38) have carried out an operation which they believe destroys the first part of the "ascending trigemello-thalamic path." Reviews of trends in surgical procedures have been presented by various authors (6, 7, 13, 20, 40). Most recent papers, in discussing tractotomy, state that the procedure has many advantages over root section but, because of a few disadvantages, is not likely to replace the Spiller-Frazier operation. Since the approach is the same as that for the Dandy operation for root section, and is somewhat simpler, tractotomy may be the method of choice where the occipital approach is used. Even though

tactile sensibility is somewhat diminished by tractotomy, residual tactile sensation is so nearly normal that most patients are not aware of any postoperative difference.

Summary

By means of the oscillograph, tactile impulses were traced into the spinal tract of the fifth nerve of the cat. Impulses originating in the ophthalmic division of the trigeminal were recorded as far as the caudal part of the first cervical segment, those from the maxillary area as far as the rostral part of the first cervical segment, and those from the mandibular area were followed to the junction of the medulla and cord.

Synchronous action potentials evoked electrically from a large branch of each of the divisions of the fifth nerve were recorded from the spinal tract and traced to approximately the same levels as given above. These potentials were easier to trace than the random tactile responses but could not be detected an appreciable distance below the levels mentioned. Physiological evidence is presented to support previous anatomical findings regarding the relative position of the three trigeminal divisions within the spinal tract. The ophthalmic fibers assume a ventral position, the mandibular fibers a dorsal position, and the maxillary fibers an intermediate position. Each division remains relatively independent throughout its course.

The action potential evoked electrically consisted of one or two waves each of which had one or more crests. The first wave was complete within 2 msec. and very likely resulted from activity in first order neurones. The second wave started at about 2 msec. lasted to as long as 4 or 5 msec., and evidenced activity in second or higher order neurones.

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EFFECTS OF INTENSITY AND WAVE LENGTH ON DRIVING CORTICAL ACTIVITY IN MONKEYS

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(Received for publication August 11, 1942)

INTRODUCTION

IN A PREVIOUS paper we (3) have reported a driving effect upon the electroencephalogram (E.E.G.) of the unanesthetized monkey produced by intermittent photic stimulation of the retinae at various frequencies by flashes of relatively white or neutral light. It was found to be present on an average of 55 per cent of the time for the entire group and ranged from 35 to 78 per cent in individual monkeys when flashes of 120 footcandles were employed.

It is clear from the fact that we obtained these results without anesthesia, that coincidental intrinsic and extrinsic environmental factors did not greatly disturb the monkey or significantly interfere with its cortical activity. In fact the responsiveness of the animal has augmented our hope that the driving effect may be used as an indicator of cortical reactivity even in man. In order to ascertain the range of conditions under which the driving effect may safely be employed as an indicator of cortical reactivity, it is necessary to determine its dependency upon such factors as intensity of flash, wave length of flash, flash frequency and light dark ratio. Quantitative evidence is presented for the first two of these variables in the present report.

METHOD

Experiments were carried out on eight adult monkeys (*Macaca mulatta*). The procedure and apparatus‡ for securing simultaneous electroencephalograms from both occipital regions and a record of flash intervals have been described by us elsewhere (3).

Binocular stimulation was carried out with the unanesthetized monkey in a darkened, electrically shielded cage. Light from a tungsten source was interrupted at various frequencies by means of an interposed episcotister driven by a DC motor controlled by a rheostat. A light-dark ratio of 1/1 was employed throughout. Two convex lenses and a plane mirror were employed to condense and direct the light from its source to the surface of either neutral or monochromatic filters which were placed individually into a filter holder located at a distance of 25 cm. from the eyes of the monkey. An optical image was thus formed in the plane of the filter with the light diverging from that point to the eyes of the animal. In each experiment both pupillary and accommodative reflexes were eliminated by instillation of 0.5 per cent solution of scopolamine hydrochloride into the conjunctival sacs of both eyes. Stimulation was never begun until mydriasis was complete. Blinking movements were eliminated by continuous use of lid retractors during stimulation; a bit was employed to prevent head movements.

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* The animals employed in this investigation were purchased by funds granted to one of the authors (Walker) by the Ella Sachs Plotz Foundation.

† The authors wish to acknowledge the technical assistance of Orien Woolf.

‡ The authors are indebted to Dr. Theodore Case for use of the electroencephalographic equipment and to his assistant, Mr. Samuel Adams, for aid in solving various technical problems which arose in connection with apparatus.

Results

Effect of intensity. In determining the effect of variations in intensity upon driving, the following sequence of intensities was employed with two monkeys: 2, 4, 6, 8, 10, 20, 40, 60, 80, 100 and 120 footcandles. Data on three additional monkeys were obtained at 4, 40, 80 and 120 footcandles. The sequences were not reversed due to the relative slowness of dark adaptation. For each intensity value, flash frequency was either raised or lowered through 20 steps, the range of variation being in every instance from 3 to 12.5 flashes per sec. Each flash frequency was employed for 15 seconds for each intensity (tape speed was 3 cm. per sec.). Quantitative analysis was made of all records. Driving was considered present in any frequency when five consecutive

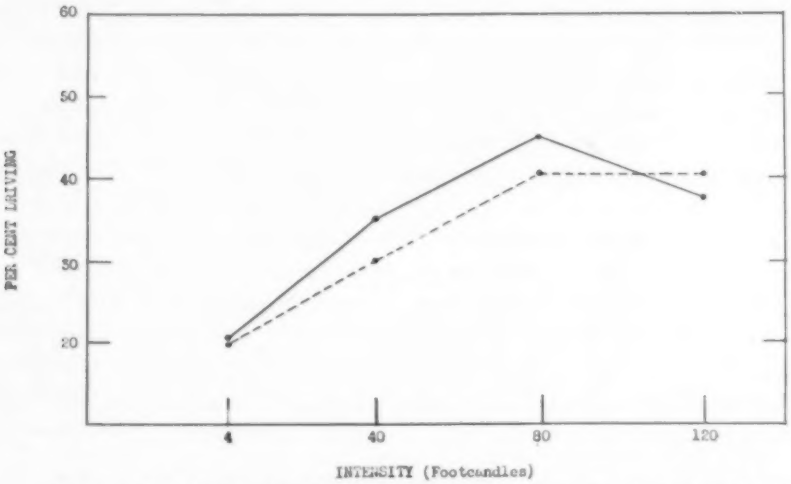

FIG. 1. Graph showing the average effectiveness in producing driving of the electroencephalogram in five monkeys by variations in intensity of intermittent flashes. Solid line—left occipital lead; broken line—right occipital lead.

waves of the E.E.G. coincided with the flash frequency. In many records the cortical frequencies followed the flash frequency throughout an entire period of photic stimulation at one intensity. The driving for each intensity was calculated in terms of the number of flash frequencies which produced driving out of the total of 20 frequencies employed. These values plotted against intensity are the basis for the curves in Fig. 1.

The results obtained for the two occipital regions are plotted separately. It will be noted that in general the two curves are quite similar and that both reach a maximum in the region of 80 footcandles. Flashes of neutral light at this intensity are about twice as effective in producing driving as four footcandle flashes. The explanation for this is not apparent. This intensity (80 footcandles) is well below the saturation point for discriminable brightness in man and presumably so for the monkey.

Effect of wave length. The effect of wave length upon driving was determined on seven monkeys (a total of 11 records) using the same optical set-up

as that employed for studying the influence of intensity except that monochromatic filters were substituted for neutral filters and the intensity was maintained at four footcandles throughout the experiment. All photometric determinations were made by means of a commercial model GM Photometer (GM Laboratories, Inc., Chicago, Illinois). This photometer consists of a galvanometer and a photo-electric cell with limiting filters to exclude all wave lengths but the visible spectrum for the human eye (sensitivity: 0.1 footcandle). Since the monkey and the human eye have been shown to have essentially similar spectral sensitivity curves (2) the photic stimuli were

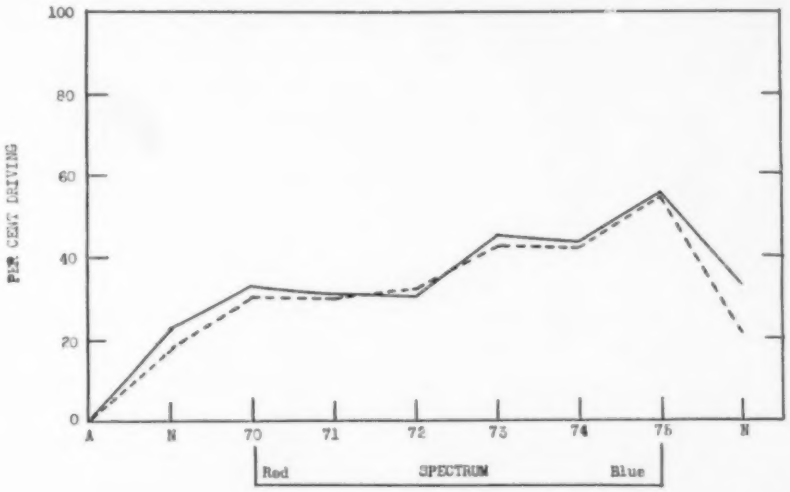

FIG. 2. Graph showing the average effectiveness in producing driving of the electrocencephalogram in seven monkeys (11 records) by variations in wave length of intermittent flashes. Solid line—left occipital lead; broken line—right occipital lead; A—auditory stimulus alone (control); N—neutral filter at four footcandles; 70–75—Wratten monochromatic filters equated at 4 footcandles.

equated for luminosity rather than for energy. The following sequence of stimulation was employed: an auditory control period, a neutral filter, Wratten monochromatic filters Nos. 70, 71, 72, 73, 74, 75, the neutral filter. The sequence of color filters and of flash frequencies was systematically alternated in the different experimental sessions for some monkeys. The per cent driving for each stimulus condition was calculated as for variations in intensity. These values plotted against their respective filters are the basis for the curves shown in Fig. 2. The transmission of each of the color filters employed is shown in the table given on the next page.

The relative effectiveness of various parts of the spectrum in producing driving is shown by the curves plotted in Fig. 2. The per cent driving for each occipital lead is plotted separately. Again it may be noted that the two curves are quite similar and that both reach a maximum in the blue region of the spectrum. In spite of considerable variation in the absolute percentage of driving for each occipital lead from one animal to another, it is apparent that

Table 1. Transmission of Wratten filters (1).

Filter no.	Range of transmission	Transmission maxima
70	635–700 mμ	700 mμ
71	600–700 mμ	640 mμ; 700 mμ
72	585–700 mμ	610 mμ; 700 mμ
73	575–700 mμ	585 mμ; 700 mμ
74	510–570 mμ	530 mμ
75	455–535 mμ	490 mμ

in general, the blue end of the spectrum tended to be more effective in producing driving than the red end. If we express this difference in terms of the extremes of our spectrum, blue filter No. 75 produced an average driving of 55 per cent as compared with red filter No. 70 which produced an average driving of 31 per cent, a difference which is quite reliable.

Relative driving effect of colored versus neutral light. The average per cent driving produced by all six of the colored filters was 38 per cent as compared with 23.5 per cent for the neutral filters. In other words, the relatively monochromatic light was approximately 1.6 times as effective in producing driving as neutral light of the same luminous energy.

Effect of temporal sequence on driving. Reversing the sequence of the monochromatic filters did not alter significantly the relative driving effectiveness of any of them. A slight general tendency for increased driving during the 45-minute experimental period is suggested, however, by the fact that neutral light was somewhat more effective on the average in producing driving at the end of the period than towards the beginning. The average of the two occipital leads gave 20 per cent driving in the second 5-minute interval and 27 per cent driving in the ninth 5-minute interval in which neutral light was used.

Summary

From an investigation of the effects of intensity and wave length on driving the cortical activity in monkey (*Macaca mulatta*) it was found that: (i) the greatest driving was obtained for variations in intensity with flashes of 80 footcandles. At this value flashes were approximately twice as effective in producing driving as flashes at 4 footcandles; (ii) monochromatic light is relatively more effective (1.6 times) in producing driving than neutral light of the same luminous energy; (iii) the blue region of the spectrum was considerably more effective in producing driving than the red region.

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REFLEX DISCHARGES IN BRANCHES OF THE CRURAL NERVE

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(Received for publication September 3, 1942)

THIS PAPER describes reflex discharges into those branches of the crural (femoral) nerve which supply the quadriceps extensor and sartorius muscles. The discharges were evoked by stimulation of dorsal roots and were conditioned by the stimulation of other dorsal roots and nerves of the ipsilateral hind limb.

The discharges in the nerve to the quadriceps, in particular its vastus internus portion, were studied because of the function of this muscle as the principal effector organ of the knee-jerk (8). In view of the fact that the knee-jerk has been described as a reflex of brief central latency which is immediately inhibited by stimulation of nerves in the ipsilateral hind limb (9, 1), it was of interest to examine the activity of its component neurons in the light of the newer observations on central reflex times and spinal inhibition (6, 7, 3). In the cat most of the motoneurons innervating the vastus internus lie in the fifth and sixth lumbar (L5 and L6) segments of the spinal cord, and the excitatory afferent axons for the knee-jerk pass into the cord via the dorsal roots of these segments, particularly L6 (8). The well-known inhibition of the knee-jerk by stimulation of such a nerve as the ipsilateral peroneal or hamstring (9, 1) involves impulses in sensory fibers, many of which enter the cord over the L7 dorsal root.

The axial distribution within the cord of the motoneurons which supply the sartorius overlaps that of the quadriceps motoneurons, the sartorius pool being shifted a little in the cephalic direction (8), and the motor fibers to both muscles pass peripherally in the crural nerve. In contrast with the action of the vastus internus as an extensor of the leg, the principal role of the sartorius is to flex the thigh. It therefore seemed of interest to compare the reflex discharges into the nerves of these two muscles which have entirely different functions; but whose motoneurons are in topographical association.

METHODS

The experiments were performed on cats which had been narcotized with pentobarbital sodium ("Nembutal") or Dial (Ciba). The branches of the crural nerve which supply the vastus internus and sartorius muscles were cut distally and placed on recording electrodes for monophasic recording. The remaining branches of the crural nerve were severed, as were the obturator nerve and the iliopsoas muscle. After a laminectomy had been performed, the necessary dorsal roots were cut intradurally and placed on stimulating electrodes. The shocks delivered to the dorsal roots were usually of a strength two to three times the threshold for their most excitable fibers. The usual differential amplifier, oscillograph, and stimulating apparatus were used.

Results

1. *Difference between reflex discharges into motor nerves supplying vastus internus and sartorius muscles.* Under the conditions of the present experiments, the reflex discharges into motor branches supplying the sartorius differed considerably from those in branches to the vastus internus and other components of the quadriceps. Furthermore, the reflex discharges to the two muscles were conditioned in different ways by antecedent stimulation of the same groups of sensory axons. These differences, which existed despite the

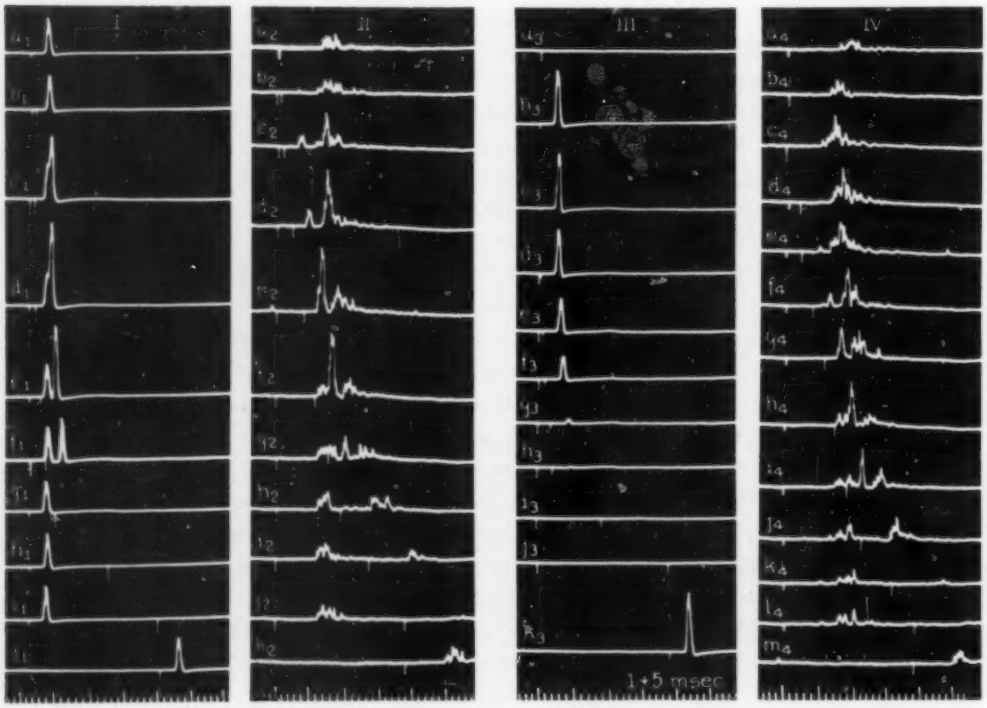

Fig. 1. Comparison of reflex discharges into the nerves to the vastus internus (columns I and III) and sartorius (columns II and IV) muscles. Columns I and II show the responses to one and two L6 dorsal root volleys. Columns III and IV show the responses to an L7 dorsal root volley (records *a*); an L6 volley (records *b*, *k* 3, *m* 4; and to an L7 volley plus an L6 volley (records *c* 3–*j* 3 and *c* 4–*l* 4). The amplification for the records of column III was *ca.* 1.7 times, for columns II and IV five times, that for column I.

overlapping axial distribution of the motoneurons supplying the two muscles (8), are illustrated by the records of Fig. 1. The discharges shown in columns I and III were recorded from the nerve to the vastus internus; those in columns II and IV from a branch to the sartorius. The motor discharges were initiated by L6 dorsal root stimulation and were conditioned by L6 dorsal root volleys (columns I and II) and L7 dorsal root volleys (columns III and IV).

A nearly synchronous discharge of brief central latency was evoked in

the vastus internus nerve by an L6 dorsal root volley (records *a* 1 and *j* 1). Records *b* 1 to *f* 1 reveal that an L6 volley facilitated the motoneurons in question to a second L6 volley for a brief period of only 2 to 3 msec. There followed a prolonged period of profound inhibition, the beginning of which is illustrated in records *g* 1 to *i* 1. In contrast, the reflex discharge set up in the nerve to the sartorius by a L6 dorsal root volley had a different nature (record *a* 2). It was characterized by impulses of relatively long central latency. In this preparation no impulses of short central reflex time appeared in response to a single L6 dorsal root volley (compare record *a* 2 with *a* 1). The responses to two L6 dorsal root shocks showed that the arrival of an L6 volley at the cord facilitated the sartorius motoneurons for *ca.* 10 msec.—considerably longer than it facilitated the vastus internus motoneurons. A prominent feature of the conditioned discharge was the appearance of impulses of short central latency. At longer shock intervals the discharges to the second L6 volley were inhibited (*cf.* records *i* 2 and *j* 2), but not as profoundly nor for as long as in the case of the vastus internus motoneuron discharge.

Further differences between the responses of vastus internus and sartorius motoneurons appeared when the reflex discharges to L6 volleys were conditioned by L7 dorsal root volleys (columns III and IV). Afferent impulses in the L7 dorsal root produced no conspicuous discharge of vastus internus motoneurons (record *a*3). The only patent effect on the discharge initiated by a subsequent L6 volley was inhibitory (records *d* 3 to *j* 3). The inhibition was profound and prolonged, and the latency of its onset very brief (cf. record *d* 3). On the other hand, an L7 dorsal root volley produced a discharge of sartorius motoneurons (record *a* 4). This discharge was similar to that produced by an L6 volley (records *b* 4, *m* 4, *a* 2 and *k* 2). Furthermore, an L7 volley facilitated the response to a subsequent L6 volley for a period of over 12 msec. At longer L7-to-L6 shock intervals, the discharge produced by the L6 volley was inhibited.

Figure 2 shows the results of two additional experiments in which the discharges into the sartorius nerve were examined. In the experiment of column I the discharge of record b_1 was produced by an L7 dorsal root volley, of records a_1 and i_1 by an L6 dorsal root volley, and of records c_1 to h_1 by an L7 plus an L6 volley. The results were similar to those shown in column IV of Fig. 1—both an L6 and an L7 volley produced relatively prolonged discharges of long central latency, and the discharges produced an L6 volley were facilitated when the L6 volley followed the L7 volley by 0–10 $\pm$ msec. An additional fact was revealed in the experiment from which the records of column II were taken. In this experiment the testing shock stimulated the fibers of the L5 as well as the L6 dorsal root. The discharge evoked in the sartorius nerve by their combined stimulation (record *b*2) showed in addition to discharges of relatively long central latency, a discharge of short central reflex time—about 1.0– msec. This discharge, like the discharges of short central latency into the nerve of the vastus internus,

was inhibited by an L7 dorsal root volley which preceded the L6 dorsal root volley at short intervals of less than 1.8 msec. (records *d* 2–*f* 2). Unlike the impulses in the vastus internus nerve, however, this sartorius motoneuron discharge of short central latency was clearly facilitated when the shock to the L7 dorsal root preceded the L6 shock by 2 to *ca.* 12 msec., as is shown by record *g* 2 for a shock interval of 2.2 msec.

These characteristics of the discharges in the nerves to the vastus internus and sartorius appeared consistently in a series of preparations. The

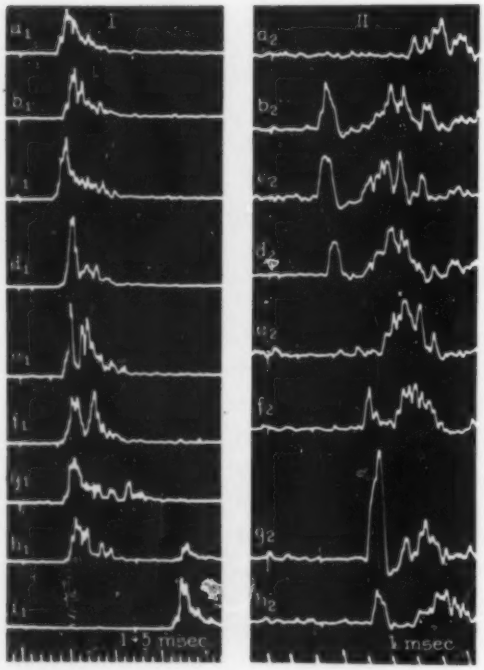

FIG. 2. Reflex discharges initiated in the nerve to the sartorius by ipsilateral dorsal root volleys; records *b* 1 and *a* 2 by an L7 dorsal root volley; records *a* 1 and *i* 1 by an L6 dorsal root volley; records *c* 1–*h* 1 by an L7 followed by an L6 dorsal root volley; records *b* 2 and *h* 2 by a volley in L5 and L6 dorsal roots; records *c* 2–*g* 2 by an L7 volley followed by an L5 plus L6 volley.

constancy of the discharges stands in contrast with the relatively variable reflexes which are recorded from ventral roots in comparable preparations. An explanation for this difference lies in the fact that a ventral root contains axons passing to several different muscles. A mere quantitative difference between the relative sizes of the discharges passing to such a muscle as the sartorius on the one hand, and to such a one as the vastus internus on the other, would explain the variability which is experimentally observed in the ventral root discharges from a series of similar preparations.

2. *Excitation and inhibition of motoneurons supplying the quadriceps (vastus internus).* The data of Fig. 3–5 have been taken from typical experiments to demonstrate (i) the central reflex times for discharges evoked in the nerve to the vastus internus by stimulation of the L6 (also L5) dorsal root, and (ii) the time course of the inhibition of this discharge as a consequence of conditioning volleys in the L7 dorsal root.

In the experiment from which the records of Fig. 3 have been taken, a single L6 dorsal root volley evoked a sizable reflex discharge into the nerve of the vastus internus (oscillogram *b*). The discharge represented a slightly dispersed volley of impulses, the central latencies for which were determined by calculation of the times for conduction in afferent and efferent fibers. Record *c* shows the potential changes which were set up as a result of the application of a shock to the L6 dorsal root and recorded at an electrode placed on the

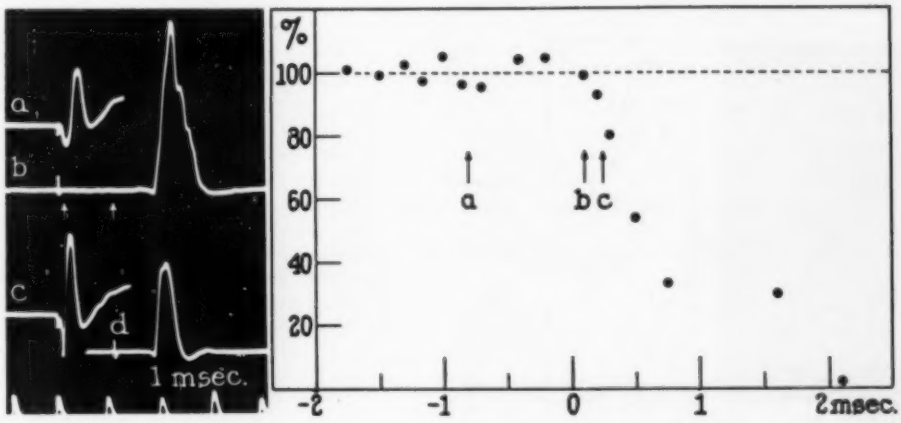

FIG. 3. Inhibition of the reflex discharge in the nerve of the vastus internus by an L7 dorsal root volley. The testing stimulus which evoked the reflex was an L6 dorsal root volley.

$$\text{Ordinates: } \frac{\text{Average height of conditioned reflex}}{\text{Average height of unconditioned reflex}} \times 100.$$

Abscissae: Interval at which the shock to the L6 dorsal root followed the shock to the L7 dorsal root. The significance of the arrows is described in the text. Oscillograms: *a*, the potential changes which were evoked at the dorsal surface of the L6 segment of the cord as a consequence of the delivery of a shock to the L7 dorsal root; *c*, same, due to the L6 dorsal root volley; *b*, the unconditioned reflex discharge set up in the nerve to the vastus internus by delivery of a shock to the L6 dorsal root; *d*, response at the recording electrodes on the nerve to the vastus internus due to direct electrical stimulation of the motoneurons at the L6 segment of the cord.

dorsum of the cord at L6. An indifferently placed electrode completed the recording circuit. Oscillogram *a* is a similar record obtained in response to stimulation of the L7 dorsal root. Conduction time in the dorsal roots was measured as the interval between the stimulus escape and the moment at which the oscillograph spot started to rise to the base-line from the first positive (downward) trough of the primary spike of the cord potential (2). An electrical shock was then delivered directly to the motoneurons of the L6 ventral horn and the resulting motor discharge recorded at the electrodes on the nerve to the vastus internus (record *d*). The shock-response interval for such an *m* wave (4, 5) has been shown to be a valid measure for conduction time in the motor fibers of a reflex arc (6, p. 377).

Utilizing these corrections for conduction times in the L6 dorsal root and in the motor fibers, it is seen that the afferent volley arrived at the L6 segment of the cord at the time of the first arrow (record *b*) and that the first reflex impulses were initiated in the motor axons at the time of the second arrow. The interval between the arrows—0.95 msec.—represented the minimal central latency for the reflex discharges. Minimal central reflex times of 0.9 to 1.6 msec. were obtained in other experiments. The shortest times presumably represented discharges in two-neuron reflex arcs.

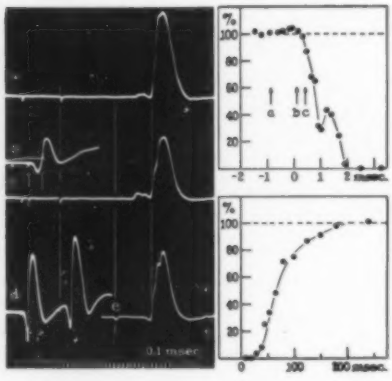

FIG. 4. Same as Fig. 3, except that the tested reflex was evoked by the second of two L6 dorsal root volleys. Oscillograms: *a*, unconditioned testing response; *c*, conditioned testing response; *b*, potential changes at the dorsum of the cord at the L6 segment due to a conditioning (L7) dorsal root volley; *d*, same, due to the two L6 dorsal root volleys; *e*, "*m* wave" at the recording electrodes on the nerve to the vastus internus due to direct electrical stimulation of the motoneurons at the cord.

The graph of Fig. 3[1] demonstrates the effects of a conditioning L7 dorsal root volley on the reflex discharge shown in oscillogram *b*. The conditioning was inhibitory and the latency of the inhibition was brief; indeed, it was already evident at a conditioning-shock to testing-shock interval of 0.2 msec. Making corrections for the conduction of impulses in the L6 and L7 dorsal roots and in the motor fibers to the vastus internus, it turns out that the arrow *a* marks the shock interval (L7 following L6 by 0.8 msec.) at which the tested impulses were set up in the motor axons just at the moment when the conditioning L7 dorsal root volley arrived at the cord. For points on the graph to the left of arrow *a*, the conditioning impulses arrived at the cord after the tested reflex impulses had been set up and passed peripherally into the motor axons; therefore a conditioning effect could scarcely have been expected. For points on the graph to the right of arrow *a*, the conditioning impulses arrived before the tested motoneurons fired. At the shock interval indicated by the arrow *b*, the conditioning and testing dorsal root

[1] In the graph of this and subsequent figures, the degree to which the tested discharge was altered as a result of the conditioning stimulation is indicated on the ordinate scale: and, as is customary, the abscissae represent the interval at which the testing shock followed the conditioning shock. The only unusual feature is that some of the graphs have been extended to the left of the zero shock interval to include negative values. A negative conditioning-shock to testing-shock interval merely means that the conditioning shock followed the testing shock. Because the tested motor impulses were initiated only after a latency which included both the conduction time in testing dorsal root fibers and the central reflex time, a conditioning shock could follow the testing shock and still precede the time of initiation of the tested motor impulses.

volleys arrived simultaneously at the L6 segment of the cord. The interval between arrows *a* and *b* therefore represents the central reflex time for the tested impulses. Only if the conditioning impulses arrived slightly earlier with respect to the time of initiation of the tested motor impulses (as indicated by arrow *c*), was the inhibition clearly established. Therefore the interval between arrows *a* and *c* represents the minimal time by which the arrival at the cord of the conditioning volley had to precede the firing of the tested motoneurons in order that inhibition could be observed. This interval

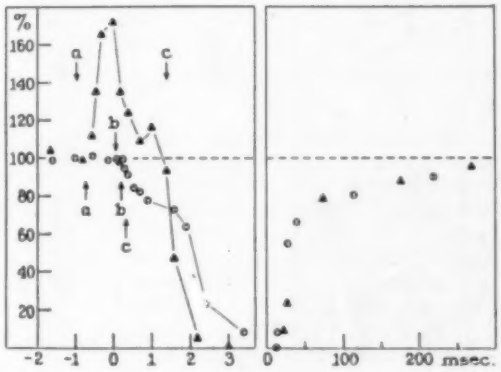

FIG. 5. Conditioning curves as in Figs. 3 and 4. The tested reflex was evoked in the nerve to the vastus internus by two L5 dorsal root volleys. ▲—conditioning by an L6 dorsal root volley; ○—by an L7 dorsal root volley. The significance of the arrows is described in the text.

may be defined as the *central inhibitory time* for the effects of the conditioning volley on the tested motoneurons. In this experiment it amounted to 1.0 to 1.1 msec. This value is about the same, or slightly longer than, that reported for the previously studied cases of the so-called direct inhibitory action of centripetal sensory and motor volleys (3, 7).

The total time course of the inhibitory action is illustrated in Fig. 4. In the preparation from which the data of this figure were taken, a single L6 dorsal root volley produced only a small reflex discharge with a minimal central reflex time of 1.6 msec. The response to the second of two L6 dorsal root volleys which were spaced at 0.95 msec. was larger and had a minimal central latency of 1.0⁻ msec. (record *a*). This facilitated motoneuron discharge was first inhibited when conditioning impulses in the L7 dorsal root arrived at the L6 segment of the cord 1.2 msec. before the firing of the tested motoneurons. As the interval between the L7 and the testing L6 dorsal root volleys increased, the inhibition became complete, subsequently to disappear only when the tested motoneuron discharge followed the conditioning volley by nearly 200 msec. (0.2 sec.). In comparable experiments the duration of the period of inhibition was not shortened after the intravenous injection of sufficient curare to abolish all muscular contractions.

The similarities and differences between the effects of L6 and L7 dorsal root volleys on the vastus internus motoneurons may likewise be demonstrated by initiating a motoneuron discharge with an L5 dorsal root volley and conditioning with L6 and L7 volleys. In the experiment from which the

data in Fig. 5 were taken, the tested vastus internus motoneuron response was initiated by the second of two closely spaced L5 dorsal root volleys. The curve marked with closed triangles was the result of conditioning by an L6 dorsal root volley; that marked with open circles was due to conditioning by impulses in the fibers of the L7 dorsal root. The arrows have the same significance as in the preceding figures. Those above the 100 per cent line relate to the L6 conditioning; those below the line to L7 conditioning. Arrows *a* mark the conditioning-shock to testing-shock interval at which the tested motoneurons fired simultaneously with the arrival of the conditioning volley at the cord, at the axial level of the boundary between the L5 and L6 segments. Arrows *b* mark the interval at which the conditioning volley arrived at the cord simultaneously with the testing volley in the L5 dorsal root; and arrows *c*, the shock interval at which the inhibition due to the conditioning impulses was first apparent. The central reflex time for the tested motor impulses (the *a*–*b* interval) was about 1.0 msec. Facilitation was produced by an L6 dorsal root volley which arrived at the cord, at the level of the boundary between the L5 and L6 segments, 0.5 ±—2.3 ± msec. before the discharge of the tested motoneurons. When the conditioning impulses arrived earlier, inhibition was apparent; it lasted until the conditioning-shock to testing-shock interval exceeded 200 msec. An L7 dorsal root volley produced only inhibitory effects, except possibly when it preceded the tested motoneuron discharge by more than 200 msec. —a postinhibitory "rebound" has been noted in some experiments (*cf.* Fig. 7). The inhibition had a central latency (central inhibitory time) of 1.1—1.2 msec.; that is, a condition for the appearance of the inhibitory action was that the L7 impulses arrive at the cord 1.1—1.2 msec. before the firing of the tested motoneurons. The degree of inhibition became greater as the conditioning-shock to testing-shock interval became longer; at still longer shock intervals it declined along the same time course as the inhibition produced by an L6 dorsal root volley.

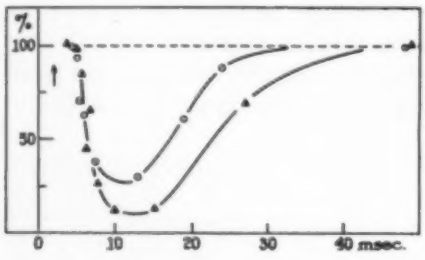

FIG. 6. Conditioning curves as in preceding figures. The tested reflex was evoked in a motor nerve to the quadriceps by two L6 dorsal root volleys. A volley in the sural nerve conditioned the response according to the curve marked (▲); a volley in a small branch to the biceps according to the curve marked (○). The arrow denotes the shock interval at which the conditioning (L7) and the testing (the second L6 dorsal root) volleys arrived simultaneously at the cord.

The inhibition of the vastus internus motoneuron discharge by the stimulation of such nerves of the hind leg as the hamstring or peroneal is similar to that produced by L7 dorsal root stimulation, provided that account is taken of the greater time required for the conduction of the centripetal volley.

Use of a conditiong volley in a small peripheral nerve, as well as of a submaximal alpha volley in a larger nerve or the L7 dorsal root, produced an inhibitory effect which had a longer latency and shorter duration than that produced by the larger volleys described above. The effect was also less profound; and because the inhibition of the testing response was not complete, it was possible to show that the inhibitory effect attained its maximal value when the conditioning impulses arrived at the cord 10–15 msec. before the discharge of the tested motoneurons. These features of the inhibitory process are shown in Fig. 6 and 7. In Fig. 6 the curve marked with open circles represents the inhibition which was produced by a volley in a small branch to a hamstring muscle. Stimulation of the sural nerve—a relatively small cutaneous nerve—produced the curve marked with closed triangles. It is seen that the stimulation of sensory fibers from cutaneous and deep parts of the hind limb had similar effects on the vastus internus motoneurons.

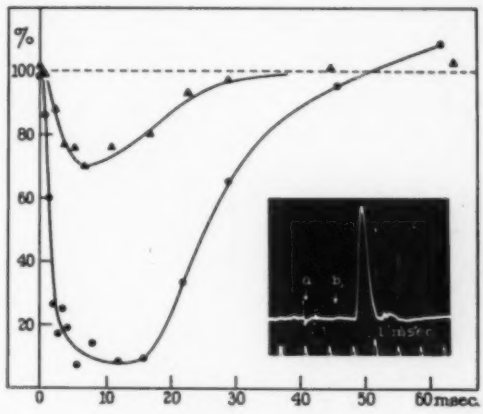

FIG. 7. Conditioning of vastus internus motoneuron discharge by impulses in sensory and motor fibers of the hamstring nerve. The inset shows the tested reflex discharge which was set up in the nerve to the vastus internus by a dorsal root volley in the L5 plus L6 dorsal root fibers. The coordinates are as in the preceding figures. The curve (●) shows the conditioning of the tested reflex by a volley in the hamstring nerve. The L7 and sacral dorsal roots were then cut. Since the L5 and L6 dorsal roots had been cut at the beginning of the experiment, the hamstring nerve was thereby deafferented. A centripetal volley in the hamstring nerve then conditioned the tested reflex as shown by the curve (▲).

Since the inhibitory effects of brief central latency have been demonstrated to be produced by centripetal volleys in motor fibers (7), it is of interest to differentiate between the effects of the sensory and the motor components of a centripetal hamstring volley up on the inhibition of vastus internus motoneuron discharges. The testing vastus internus motoneuron response shown in the inset of Fig. 7 was produced by the stimulation of the L5 plus L6 dorsal roots. As shown by the interval between the arrows, the central reflex time for the first impulses of this discharge was about 1.25 msec. The points shown as solid circles represent the conditioning effects of a volley of impulses in the alpha fibers of the hamstring nerve. It therefore represented the effects of impulses in both sensory and motor fibers. The dorsal roots of the L7 and sacral segments were then cut intradurally, leaving intact only the motor fibers of the hamstring nerve. Stimulation of the hamstring nerve then conditioned the tested vastus internus motoneuron discharge, as is shown by the points marked with solid triangles. The curve (●) therefore represents the combined effects of cen-

tripetal sensory *plus* motor impulses; the curve (▲) only the effect of motor impulses. Obviously the action of the centripetal motor impulses was a not negligible fraction of the effect of sensory plus motor impulses. Account must be taken of such actions of antidromic motor impulses in experiments in which mixed peripheral nerves are stimulated. This is especially true in view of the fact that the inhibition of quadriceps motoneuron discharges by hamstring motor volleys is not as great as the inhibition of the discharges of many groups of motoneurons by antidromic volleys in other motor axons—for instance, the inhibition of vastus internus motoneuron discharges by antidromic volleys in other motor branches to the quadriceps (7).

Discussion

The net result of an L7 dorsal root volley on the excitability of the vastus internus motoneurons, as tested in the present experiments, was a powerful inhibitory action of brief central latency and long duration. Yet it must not be supposed that stimulation of such a large and heterogeneous population of afferent fibers as comprise a dorsal root would result in the production of only inhibitory actions, direct and relayed through interneurons, on the tested motoneurons. Indeed, the conditioning curve of Fig. 4 suggests the presence of a very slight facilitation of the tested discharge previous to the onset of its inhibition; and at shock intervals of 1 to 2 msec. in the curves of Fig. 3, 4, and 5, there is a discontinuity which might be interpreted as evidence for a submerged facilitatory action of the conditioning volley. Thus one cannot state whether the observed minimal central inhibitory time of *ca.* one msec. would have been shorter had impulses in fibers responsible for an excitatory action on the tested motoneurons been excluded from the conditioning volley. At any rate, in the present experiments a conditioning (inhibitory) volley, which arrived at the cord *immediately* before the discharge of the tested motoneurons, produced no demonstrable inhibitory action. A condition for the appearance of a demonstrable inhibitory action was that the conditioning volley arrive at least one msec. before the firing of the tested motoneurons; that is, the inhibitory volley had to arrive at the cord approximately simultaneously with, or earlier than, the testing dorsal root volley which fired the tested motoneurons after a central reflex time of *ca.* one msec.

As has been pointed out (6, p. 381 *et seq.*), one cannot at present state whether central reflex times as long as one msec. pertain to 2-neuron arcs, or to 3-neuron arcs, or to both. In the present experiments the central reflex times for the tested motor impulses into the nerve of the vastus internus, and the central inhibitory times for the effect of the L7 dorsal root impulses upon them, have had durations of *ca.* one msec. and, in some experiments, even a little more. It has therefore seemed wise to be noncommittal as to whether or not the tested reflexes were strictly 2-neuron arc discharges, as well as to whether the inhibitory effect was necessarily mediated by the direct action of the dorsal root fibers on the tested motoneurons.

It must be emphasized that the results described in this paper may apply only to preparations such as those which were studied—that is, to cats under barbiturate anesthesia, with the stimulated dorsal roots and the examined motor nerves cut peripherally. The nature of the reflex discharges in the nerves of the vastus internus and sartorius might be different in other types of preparations, just as the character of the knee-jerk and the inhibitory action of a hamstring volley upon it differ greatly according to whether the preparation is decerebrated or spinal (1).

Summary

The sartorius and the vastus internus are two muscles of different function which are supplied by motor axons originating in the same and adjacent segments of the spinal cord and passing together peripherally in the crural nerve. In a series of cats under pentobarbital sodium anesthesia, reflex discharges were evoked in the motor nerves to each of these muscles by the stimulation of dorsal roots. The discharges had a constancy from preparation to preparation, which stands in contrast with the variability observed in ventral root discharges similarly evoked in comparable preparations.

In spite of the topographical association of the two groups of motoneurons, the reflex discharges in the motor branches supplying the sartorius differed considerably from those in branches to the vastus internus and other components of the quadriceps. Furthermore, the reflex discharges to the two muscles were conditioned in different ways by antecedent stimulation of the same groups of sensory axons. An L6 dorsal root volley evoked in the nerve to the vastus internus a nearly synchronous discharge having a central latency of only 0.9 to 1.6 msec. It evoked in the nerve to the sartorius a dispersed discharge of relatively long central latency. An L7 dorsal root volley produced no conspicuous discharge of vastus internus motoneurons. Its only effect on the discharge produced by an L6 volley was inhibitory. The profound inhibitory effect had a duration as long as several hundred msec. The latency of its onset was brief; it was already present when the conditioning volley arrived at the L6 segment of the cord only 1.0 to 1.1 msec. before the firing of the tested motoneurons. In contrast, an L7 volley produced in the branches to the sartorius a discharge similar to that produced by an L6 volley. The discharge to an L6 volley was facilitated by an L7 volley preceding at intervals up to *ca.* 15 msec. At longer intervals the discharge to the L6 volley was inhibited.

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ACTION POTENTIAL AND ENZYME ACTIVITY IN THE ELECTRIC ORGAN OF *ELECTROPHORUS ELECTRICUS* (LINNAEUS)

I. CHOLINE ESTERASE AND RESPIRATION

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INTRODUCTION

IT IS GENERALLY accepted that the discharge of electric organs is fundamentally identical in its nature with the action potential of ordinary nerves, the high voltage being caused by the arrangement of the electric plates in series. The close relationship between the E. M. F. of the discharge and the activity of choline esterase in electric organs suggests that acetylcholine metabolism is intrinsically connected with the action potential. This new concept of the role of acetylcholine is strongly supported by several other facts and has recently been described (4, 5, 6).

The parallelism between E. M. F. and enzyme activity first became obvious when, in different species of electric fishes, V per cm. and number of plates per cm. were compared with the concentration of choline esterase (4). It was next shown that the concentration of choline esterase (Ch.E.) in the electric organ of *Electrophorus electricus* decreases from the head to the caudal end in an S-shaped curve and that this curve appears to be similar to that in this organ for the V per cm. and number of plates per cm. (5). The electrical measurements were, however, made on other specimens than the chemical determinations. The connection of the action potential with a chemical reaction is obviously of interest and it appears therefore desirable to establish the relationship in quantitative terms, to compare it with other electrical quantities (resistance, current, power) and to determine what other chemical reactions may be connected with the action potential. In ordinary nerves the electrical changes occurring during activity are small and rapid, the E. M. F. of the action potential being only of the order of millivolts and its duration of the order of milliseconds. These changes and their rate are well within the range of electrical methods, but chemical methods available make it difficult to correlate chemical reactions with electrical phenomena. In the electric organ of *Electrophorus electricus* the voltage of the discharge is high and the differences between the different sections are great. It offers particularly favorable material for studies on the mechanism of nerve activity, especially of chemical reactions involved in electrical changes occurring during nerve activity.

* Aided by grants from the Dazian Foundation, and the Fluid Research Fund of the Yale School of Medicine.

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Such investigations have now been initiated. This paper presents observations on voltage, amperage, resistance and Ch.E. activity on single specimens. Histological findings are presented and respiratory rates are compared with Ch.E. concentration.

METHODS

a. Electrical measurements. The fish was removed from the water and the excess moisture allowed to drain from the skin, which remained, however, wet enough to insure good electrical contact. Voltages were measured with a cathode-ray oscillograph drawing no appreciable current. The terminals of the oscillograph were joined to electrodes which were strips of aluminum 1 cm. wide set transversely in a wooden trough in which the fish was laid. These electrodes made contact at desired positions along the electric organs, through several square centimeters of wet skin next to the organs. A calibrated variable resistance could be connected between the electrodes to draw current from the organs during the discharge.

A set of measurements with one position of the electrodes was made in about three minutes, during which the fish was gently prodded to make it discharge at a frequency high enough for convenient measurement but not high enough to produce rapid fatigue. The discharges of the large organs and the bundles of Sachs could be differentiated by their characteristics described elsewhere (1). The discharge of the bundles of Sachs was generally obtained with a slighter stimulus than that of the large organ; often no stimulus was required. After each set of measuremts the fish was returned to the water for 15 minutes or longer before another set was made with different positions of the electrodes.

The recorded voltage was always the peak voltage of the discharge. It was noted visually on the oscillograph calibrated with a measured voltage. When precautions against fatigue were taken as described, it was found that for any given position of the electrodes and any given value of the variable resistance there was a recurrent peak voltage. Voltages higher and lower than this value were also observed. The lower values occurred more often than the higher; consequently the recorded recurrent voltage was generally somewhat above the average voltage. Estimates of the recurrent voltage by different observers would agree within 5 or 10 per cent. In a set of readings with one position of the electrodes, peak voltages were recorded with different values of the variable resistance. The first reading of the set, and also the last, for a check, were made with the circuit open, so that no current was drawn outside the body of the fish. The readings between were made with decreasing values of the resistance so chosen that with the lowest resistance the peak voltage was about one-fourth of that on open circuit.

b. Determination of choline esterase. The activity of choline esterase was determined with the Barcroft-Warburg manometric method (5). The following solution was used as medium: to 100 cc. of 0.04 molar $MgCl_2$ 20 cc. of 0.15 molar bicarbonate were added. Since the rate of hydrolysis at this Mg concentration[1] is as high as with Ringer concentration this medium appeared preferable. It not only can be more quickly prepared, but can be kept for a long time since no precipitation occurs.

The importance of thorough homogeneous grinding of the highly active tissue has been emphasized (5). Since a large number of determinations had to be made in the shortest possible time, mechanical grinding with a homogenizer (7) has been used except in the experiments with specimens No. 1 and 5, where the tissue was ground by hand. The mechanical grinding has to be done with great care. Silicate has to be added. The experiments and controls made on specimen No. 2 indicate that the pieces should not be too great (see below p. 506). After grinding the suspension was diluted, and the amounts of tissue used per vessel generally did not exceed 0.5–1.0 mg. according to the enzyme activity for reasons previously discussed. Only in the experiments with the bundle of Sachs larger amounts were taken. Weighing and manometric readings were carried out in the same way as before (5).

c. Respiration. Oxygen uptake was measured by the usual Barcroft-Warburg manometric method. 0.1 molar phosphate buffer of pH 7.4 was taken as a medium. If the substrate was glucose or pyruvate, slices of finely minced tissue were used in conical vessels,

[1] Nachmansohn. Unpublished experiments

and 0.2 cc. of 8 per cent KOH was put in the center well. It is difficult to cut the gelatinous tissue in thin slices, but since respiration is extremely small, diffusion of O_2 through the finely minced tissue most probably is not the limiting factor.

The oxidation of succinate was measured with homogenized tissue. Square vessels with a side bulb were used. The substrate concentration was 0.15 molar in 0.1 molar phosphate buffer of pH 7.4. In the side bulb were put 0.1 cc. of cytochrome c solution, containing about 1.5 mg. of cytochrome c in phosphate buffer. The cytochrome was prepared according to the method of Keilin and Hartree. It was added after thermoequilibrium was reached. Controls were run without succinate, and only the difference between the oxygen uptake of control and experiment recorded.

Succinic dehydrogenase was determined with the manometric method according to the principle used by Quastel and Wheatley (8). Ferricyanide is reduced by the hydrogen of succinic acid. For one reduced molecule of ferricyanide one molecule of acid is formed and in bicarbonate solution this gives rise to one molecule of CO_2. The details of the method as used in these experiments were similar to those used by Nachmansohn and Steinbach (6). The concentration of sodium succinate was 0.15 molar.

d. Histological preparations. All fishes were killed by freezing in dry ice. Immediately after death, blocks were cut from the frozen organ. The length of the blocks in the direction head to caudal end was usually exactly one cm. From specimen No. 2 blocks 2 cm. long were cut. The blocks were cut out in most cases close to the center of the segment of which the voltage had been determined. Serial sections were made using Zenker fixation and Mallory aniline blue stain.

Results

The experiments were carried out on 4 specimens, two small and two medium sized. On a fifth specimen, of medium size, only Ch.E. determinations were made. But since the total maximal discharge was known and differs from that of the two other medium sized specimens, the description appears useful and has been added.

The lengths of the specimens and their organs were as follows:

Specimen No.	Total length	Length of the electric organ
	cm.	cm.
1	51	41
2	57	47
3	112	92
4	114	94
5	105	87

A. *Electrical data*

A complete series of measurements on the discharge of the large organ made on specimen No. 4 is recorded in Table 1. In the first set of the series with the electrodes placed at 0 and 10 cm. distance from the head end of the electric organ there was reason to think that the fish had become fatigued during its exertions, muscular and electrical, while it was being removed from the tank and placed in the trough. This set was repeated therefore after the fish had rested half an hour in the water, as well as at the end of the series.

The recorded values of the resistance were known from the calibration. Those of the peak voltage were measured as already described. The current

Table 1. Measurements of the discharge of the large organ. Specimen No. 4. Ω = ohms, V = volts, A = amperes. ∞ = open circuit.

Position of electrodes	0–10 cm.		0–10 cm.		20–30 cm.		40–50 cm.		50–60 cm.		0–10 cm.	
Ω	V	A	V	A	V	A	V	A	V	A	V	A
∞	64	0	65	0	38.6	0	11.5	0	8.3	0	59	0
800	60	0.075	59	0.074	34.5	0.043	10.0	0.0125	7.1	0.0089	52	0.065
400	47	0.118	48	0.120	31.2	0.078	7.9	0.0198	6.4	0.0160	46	0.115
200	27	0.135	42	0.210	26.0	0.130	6.0	0.030	5.1	0.0255	37	0.185
100	18	0.180	31	0.310	18.1	0.181	3.9	0.039	3.7	0.037	25	0.250
50	7	0.140	17	0.340	10.1	0.202	2.4	0.048	2.4	0.048	16	0.320
∞	56	0	59	0	38.6	0	12.0	0	8.6	0	57	0

produced in the resistance at the peak of the discharge is the quotient of the voltage by the resistance.

The data are shown graphically in Fig. 1, in which the voltage is plotted as ordinate against the current as abscissa and a straight line is drawn through the plotted points, as was done by Coates and Cox (2) on *Torpedo occidentalis*. The intercept of this line on the axis of voltage is taken as the maximum voltage for each segment of the organ and the intercept on the

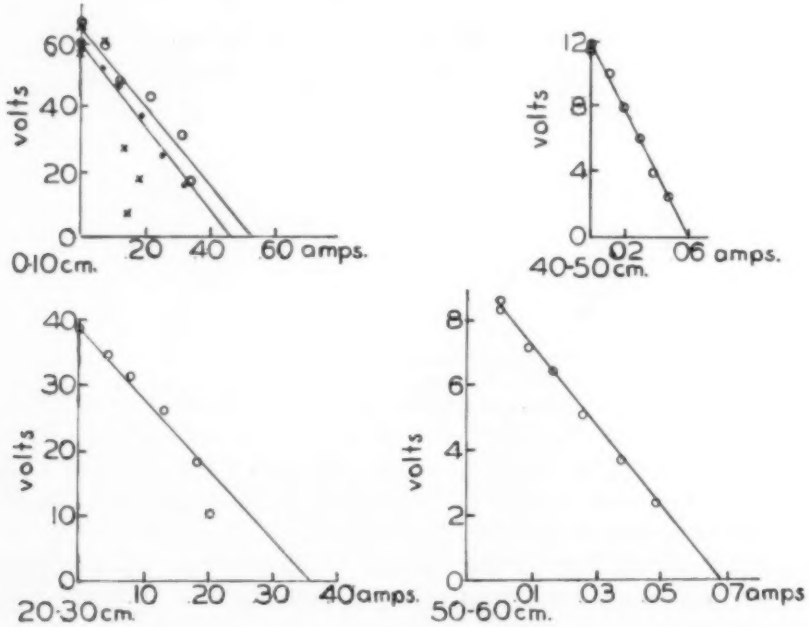

FIG. 1. Voltage of the discharge of the electric organ recorded with four different positions of electrodes. Abscissae: amperes. Ordinates: volts.

axis of current as the maximum current. All the values of maximum voltage and current recorded in Table 2 were similarly obtained. The values of resistance recorded in this table are the quotients of the maximum voltage by the maximum current.

The higher the resistance, the steeper is the line through the points.

Table 2. Electrical data recorded on specimens No. 1–4.

Specimen No.	1. Position of electrodes (cm)	2. Maximum voltage (V)	3. Maximum current (A)	4. Resistance (Ω)	5. Voltage per cm.	6. No. of electroplaxes per cm	7. mV per electroplax	8. QCh.E. average	
1.	1–6	95	0.148	640	19.0	133	145	492	
	11–16	79	0.104	760	15.8	119	133	415	
	16–21	44	0.059	750	8.8	72	119	280	
	21–26	20.4	0.019	1060	4.1	—	—	150	
2.	1–6	126	0.307	410	23.9	146	164	455	
		113	0.257	440					
	11–16	92	0.278	330	18.4	131	140	407	
	21–26	45	0.128	350	9.0	—	—	320	
	31–36	12	0.021	570	2.4	—	—	225	
3.	0–10	60	0.374	160	6.7	—	—	145	
	20–30	34	0.243	140	3.8	48	79	81	
	40–50	9.2	0.077	120	1.0	—	—	60	
	50–60	7.0	0.041	170	0.8	—	—	—	
4.	0–10	65	0.52	125	6.9	65	106	110	
		59	0.46	128					
	20–30	39	0.36	108	4.3	40	107	81	
	40–50	11.9	0.060	198	1.3	—	—	51.5	
	50–60	8.5	0.068	125	0.9	—	—	—	
	30–40	7.3	0.028	260	0.81	—	—	—	b.o.S.
		8.6	0.043	200					
	40–50				0.91	—	—	21	
		7.8	0.035	220					
	60–70	8.1	0.051	160	0.90	8	112	23	

With fatigue the points lie on a steeper line, so that at least one indication of fatigue is an increased resistance. With marked fatigue the points may fall so irregularly that no line can be drawn. In this case also the readings are not so well reproduced, so that the points themselves are uncertain. Such a set of points is shown by the crosses of Fig. 1 (a), plotted from the first set of readings of Table 1. The second set is shown by the open circles and the third set by the closed circles. The points of these two sets fall not far from a common line, but it seemed better to draw one line for each set. Two values

were recorded therefore for the maximum voltage, maximum current, and resistance (see Table 3, specimen No. 4). The mean of the two values of maximum voltage was used to obtain the voltage per cm. In some of the observations on other fish, when two sets of measurements were made on the same segment of the organs, and the values of voltage and current were plotted as in Fig. 1, a single straight line served equally well to describe

Table 3. Concentration of choline esterase at different sections of the electric organ.

Specimen No. 2					Specimen No. 4				
Distance from ant. end (cm.)	Fresh weight (mg.)	QCh.E.			Distance from ant. end (cm.)	Fresh weight (mg.)	QCh.E.		
		single	average				single	average	
3	311.0	435 470 450 465	*455*	Large organ	5	59.0 42.0 34.0	100, 88, 85 100, 98 139	91.0 99.0 139.0	*110.0*
11	238.0	414 435	*425*		25	99.8 36.0 51.5	59, 72, 76 121.5, 110 62, 55	69.0 116.0 58.5	*81.0*
18	158.5	351 343 391	*362*		35	66.4 63.7 40.8	54.5 65.0 85.0	— — —	*68.0*
26.5	128.5	320 300	*310*		45	65.0 43.3 71.7	50.4, 51 33.5, 37.5 82, 53	50.7 35.5 68.0	*51.5*
31	17.8	250 239	*245*						
				Bundle of Sachs	45	74.3 53.5	21.6 20.0	— —	*20.8*
					60	108.0	18.7, 27.8	23.2	*23.2*
					82	87.4 77.0	21.5 27.3	— —	*24.4*

either set. In these cases only one value was recorded for the maximum voltage, maximum current, and resistance.

The current obtained as the quotient of the voltage by the external resistance is, of course, only that flowing in the resistance and does not include any current flowing through the tissue which surrounds the organ. If there were no such leakage of current, then the computed current actually would be equal to the current flowing through the electric organ, the maximum voltage would be the electromotive force of the segment of the organ included between the electrodes, and the value of the resistance found as the quotient of the maximum voltage by the maximum current would be the actual resistance of this segment of the organ at the peak of the discharge.

It was found by Cox and Coates that a number of measurements under varied conditions could be fairly well represented by treating the current flowing through the tissue around the organ as if it flowed in a single resistance joined in parallel with the external resistance. They also found evidence that the resistance of the organ itself drops sharply during the discharge, a conclusion which conforms well to Bernstein's hypothesis that the discharge occurs when one boundary of each electroplax becomes permeable to an electric current in the direction in which the discharge passes through the organ.

To estimate the resistance of the tissue around the organ and thus to reckon its effect in reducing the external maximum voltage below the electromotive force, the resistance of the part of the fish between the electrodes was measured with an ohmmeter while the fish was resting in the trough without discharging. Resistances were found as follows for the several segments; 0–10 cm., 2000 ohms; 20–30 cm., 2200 ohms; 40–50 cm., 2400 ohms; 50–60 cm., 2200 ohms. These values might be taken as those of the resistance of the tissue around the organ on the assumption that the organ itself is practically non-conducting during the discharge. With these values the scheme of Cox and Coates could be used to compute the electromotive force and corrected values of the maximum current flowing in the segment of the organ and its resistance at the peak of the discharge. The method of the correction, however, is admittedly crude and its amount in any case did not seem important. Consequently the correction was not made, and the values of the voltage per cm. correlated with the QCh.E. have been taken from the measured maximum voltage rather than from the estimated electromotive force, which would have been always somewhat higher.

B. *Concentration of choline esterase and E. M. F.*

The tissue taken for the determinations of Ch.E. concentration was cut out as close as possible to the center of each segment, for which the electrical data were recorded. Since all the fish were prepared by freezing in dry ice and kept in it until the end of the experiments there was no danger of loss of water which may increase the QCh.E. values (5).

Figure 2 shows the data obtained with specimen No. 1. It demonstrates the close parallelism between V per cm. and QCh.E. Specimen No. 2 was about the same size as the first. Both Ch.E. concentration and E. M. F. are of the same order of magnitude as in the first fish (see Tables 2 and 3). The decrease of voltage is however steeper than in the first fish and greater than would be expected from the fall of Ch.E. concentration which is slightly smaller than in the first specimen. Two remarks, however, have to be made: This was the first specimen where the mechanical grinding was used for the determinations of Ch.E. No silicate was added and pieces of tissue of 150–300 mg. fresh weight and handgrinding were about 10–15 per cent higher than the average values obtained from the large pieces ground with the homogenizer. The average QCh.E. values from three small pieces at 11 cm. distance from the

anterior end of the organ was 459 instead of 425 obtained with the large piece ground mechanically. From three pieces at 18 cm. distance the QCh.E. was 407 instead of 362. It appears probable that the value at 3 cm. distance was actually around 500. The pieces of the caudal end were smaller and the

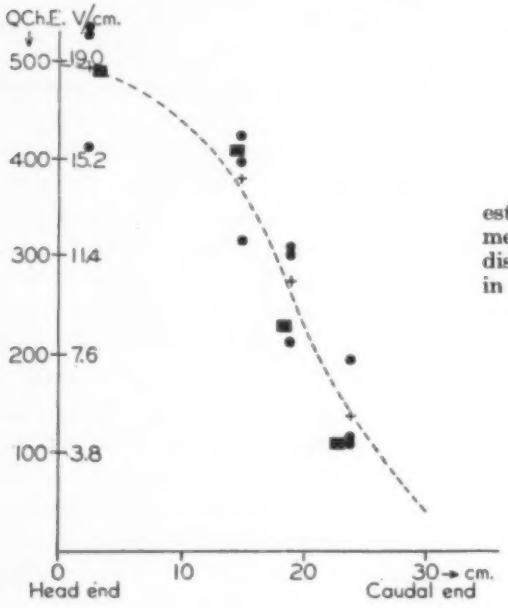

FIG. 2. Action potential and choline esterase activity in the electric organ. Specimen No. 1. Length of fish 51 cm. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E. and V/cm.
● average QCh.E. from a single piece of tissue
+ average QCh.E. values from pieces of the same section
■ V/cm.

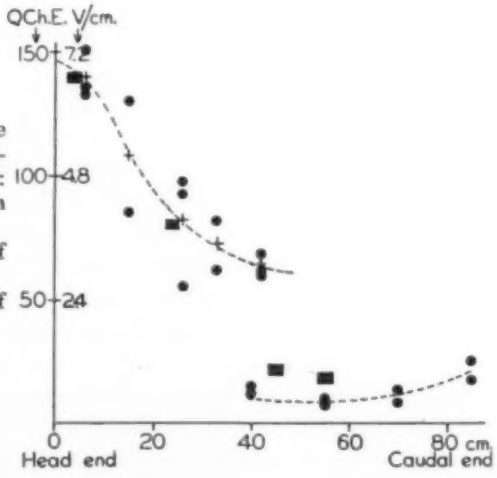

FIG. 3. Action potential and choline esterase activity in the electric organ. Specimen No. 3. Length of fish 112 cm. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E. and V/cm.
● average QCh.E. from a single piece of tissue
+ average QCh.E. values from pieces of the same section
■ V/cm.

determinations therefore probably more correct. In the experiments with specimens 3 and 4 the pieces were small (see tissue weights of specimen No. 4, Table 3). The grinding was carried out more thoroughly and silicate was added.

As far as the electrical data are concerned it must be noted that this

specimen was not in excellent condition when taken for the experiments. It had vomited repeatedly, refused food for several days, was sluggish and inactive. It seems possible that the correlation was impaired by the poor physical condition of the fish. Since Ch.E. is a stable enzyme the curve of voltage can drop below that of the enzyme concentration in case the discharge is not optimal.

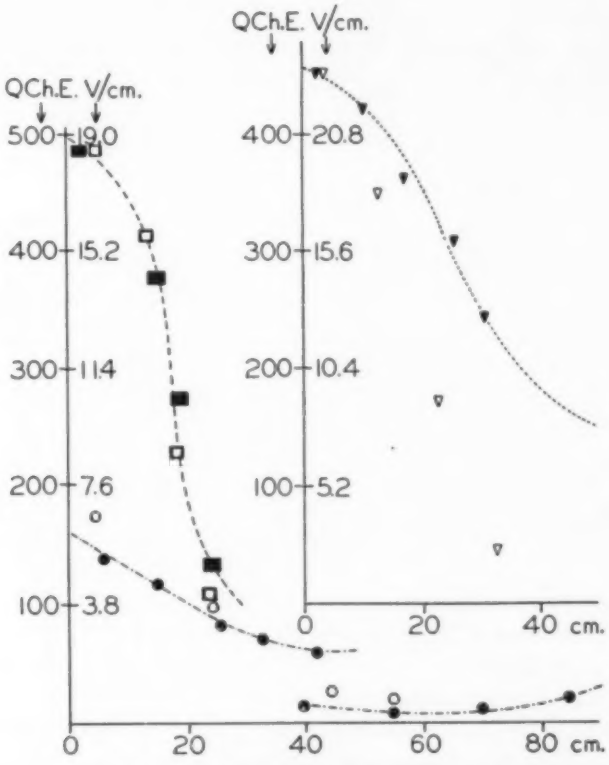

FIG. 4. Action potential and choline esterase activity in the electric organs of three specimens. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E. and V/cm.
■ QCh.E., □ V/cm. of specimen No. 1
▼ " ▽ " " No. 2
● " ○ " " No. 3

The voltage per cm. and the concentration of Ch.E. are lower in the large than in the small specimens (5). It was however not certain in these experiments whether in absolute amounts there is a correlation between voltage and enzyme concentration if different sizes are compared. The following experiments seem to indicate that there does exist a parallelism between voltage and enzyme concentration even in absolute amounts and independent of the size of the specimen.

Specimen No. 3 (Fig. 6) was a good medium sized fish. Fig. 3 shows the

data obtained in the same way as those of the first specimen in Fig. 2. In the bundle of Sachs the QCh.E. values drawn in a separate curve are much lower than in the main organ. But the concentration seems to be rather equal in its different sections. The values obtained with specimen No. 4 which had about the same size as No. 3 are practically identical with those of the third specimen (see Tables 2 and 3). On this specimen measurements were carried out on three different segments, again in agreement with the even distribution of Ch.E. in this organ.

Figure 4 demonstrates how closely, even in absolute amounts, the voltage parallels the Ch.E. activity. The data obtained on all specimens are summarized. Only No. 4 has been omitted since the figures are so close to those

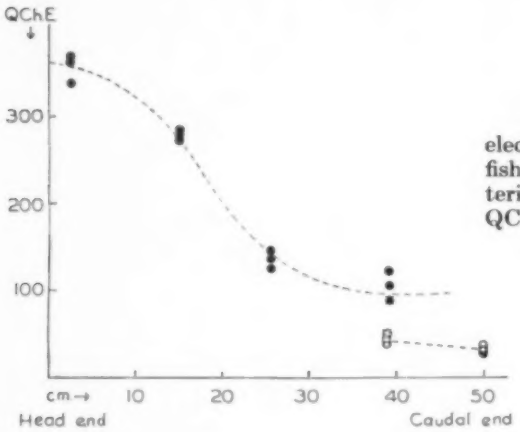

FIG. 5. Choline esterase activity in the electric organ. Specimen No. 5. Length of fish 105 cm. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E.

● average QCh.E. from a single piece of tissue

of No. 3 that they would interfere with each other. No. 2 has been drawn separately in order to make clearer the difference from No. 1, since otherwise again the two curves would interfere.

Specimen No. 5 (Fig. 7) was found dead in the Aquarium one morning. It was put immediately on ice and all Ch.E. determinations were made on the following day. It is not probable that under these conditions there was any measurable loss of water in such a short time. Figure 5 gives the values obtained. They are considerably higher than in the two other medium sized specimens, ranging from 356 (mean value of the first section, 2.5 cm. from the anterior end of the electric organ) to 106 (mean value 38 cm. from the anterior end). In the bundle of Sachs the values are 44 and 31. Even if the values are a few per cent too high, due to loss of water, it is interesting to note that they are more than twice as high as in the two other medium sized specimens. The total maximum voltage of this fish was 420 volts, as compared with less than 200 volts in specimens No. 3 and 4 (Fig. 6). This again is in good agreement with the assumption that even in absolute amounts voltage and Ch.E. activity run parallel.

The number of electroplaxes per cm. is known for many segments from the histological preparations (see paragraph D). The voltage per electroplax

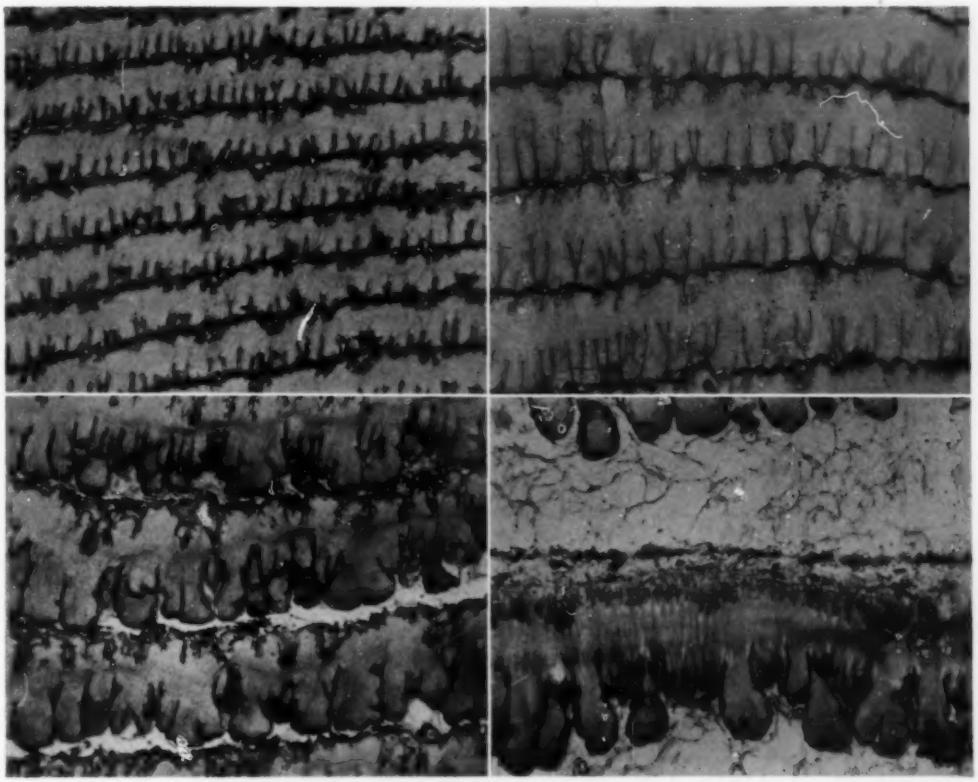

FIG. 6. Block I. Specimen No. 4. Section of electric organ 5–6 cm. from anterior end of organ. Main organ. Magnif. 145. Block II. Specimen No. 4. Section of electric organ 25–26 cm. from anterior end of organ. Main organ. Magnif. 145. Block III. Specimen No. 4. Section of electric organ 40–41 cm. from anterior end of organ. Main organ. Magnif. 145. Block IV. Specimen No. 4. Section of electric organ 55–56 cm. from anterior end of organ. Bundle of Sachs. Magnif. 145.

has been calculated in those cases where the blocks for histological preparations were taken from the center or close to the center of the segment of which the electrical data were recorded. Not enough data were available to permit an interpolation. As far as can be judged from the few data obtained on the two small specimens (Table 2, column 7) the voltage per electroplax seems to be not equal in all segments but to decrease from the head to the caudal end of the organ. The decrease of the E.M.F. may therefore depend not only on the number of plates per cm. but also on the voltage per electroplax.

In column 8 of the same table the QCh.E. values are indicated. In this case if the tissue was not cut out from the center of the segment, the values were interpolated from the curve since the data appear sufficient for such interpolation.

The voltage per electroplax in the larger specimen could be calculated only for a few cases. Both voltage and Ch.E. concentration are lower than

in the small specimens (see Table 2, column 7 and 8). Not enough data are available to permit any conclusion.

C. *Respiration*

Oxidation is the ultimate source of all energy used in living cells. The discharge of the electric organ yields a measurable amount of electric energy and, for nerve, it has been shown that activity is connected with heat

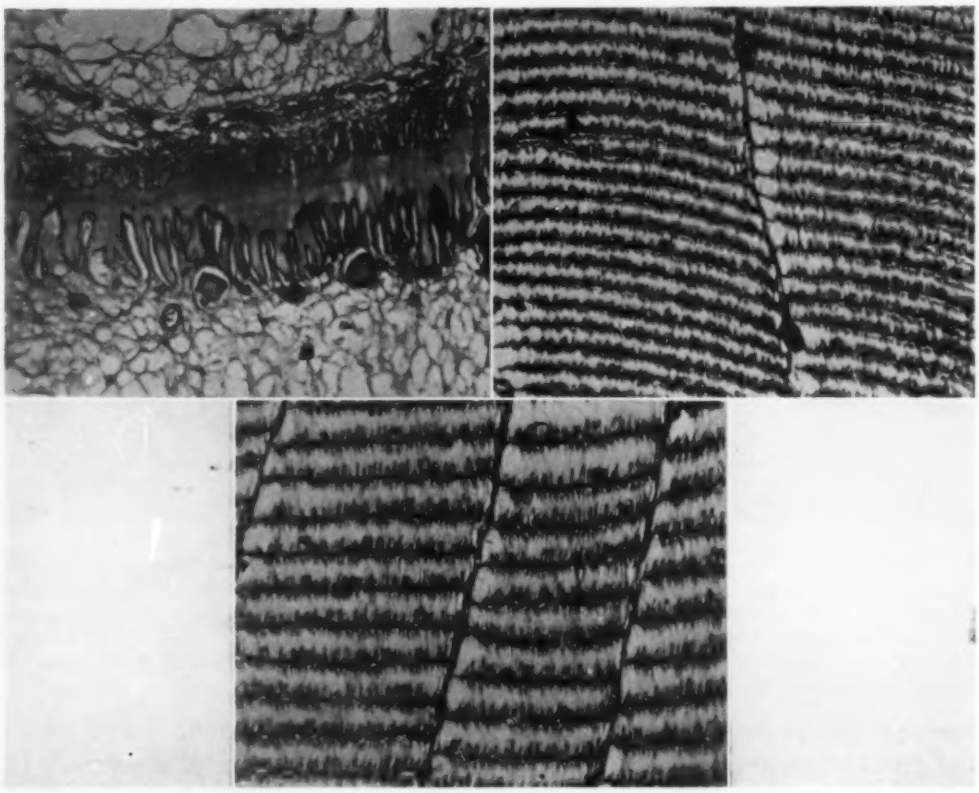

FIG. 7. Block V. Specimen No. 4. Section of electric organ 70–71 cm. from anterior end of organ. Bundle of Sachs. Magnif. 145. Block VI. Specimen No. 2. Section of electric organ 3.5–5.5 cm. from anterior end of the organ. Magnif. 145. Block VII. Specimen No. 2. Section of electric organ 18.5–20.5 cm. from anterior end of organ. Magnif. 145.

production and extra oxygen uptake. But, as recently discussed by Nachmansohn and Steinbach (6), it is improbable that oxidation is directly connected with the action potential, its energy being used only in the recovery period. Experiments by these investigators on the giant axon of squids suggest that the respiratory enzymes are chiefly concentrated in the axoplasm in contrast to Ch.E. which is localized at or near the surface of the axon.

The respiration of the electric tissue has been studied at different sec-

tions and with different substrates (pyruvate, glucose and succinate). With succinate both the dehydrogenase and the oxidizing system were examined. The tissue was taken by biopsy, since respiration is greatly impaired in fishes killed by freezing in dry ice. The fish withstands the operation well. From one fish 19 biopsies were made and it was still in rather good condition. Succinic dehydrogenase is not damaged by the freezing.

The respiration of the electric tissue is low. Table 4 shows the results

Table 4. Succinic oxidizing system and succinic dehydrogenase at different sections of the electric organ.

	Succinic oxidizing system							Succinic dehydrogenase					
Specimen No.	Distance from ant. end (cm.)	Fresh weight (mg.)	min. observ.	cmm. O_2 absorb.	cmm. O_2 per 100 mg. in 60 min. corr.	Q_{O_2} a	Q_{O_2} n	Specimen No.	Distance from ant. end (cm.)	Fresh weight (mg.)	min. obs.	cmm. CO_2 per 100 mg. corr.	µg. succ. ac. per 100 mg. in 60 min.
1	2.5	74.0	90	7.6	6.1		*−0.51*	1	2.5	62.4	75	44.2	*93.5*
	15.0	73.0	90	5.8	5.6		*−0.47*		15.0	65.0	75	43.5	*92.0*
	20.0	86.0	90	7.0	5.1		*−0.43*		23.0	59.7	90	60.6	*106.0*
3	10	219	60	8.8	3.8	−0.48	*−0.37*	3	10	111.0	110	25.8	*42*
		143	85	8.1	2.0	−0.25						25.7	
												27.9	
	30	190	85	17.6	3.1	−0.39	*−0.37*		30	110.0	120	28.2	*36*
		169	85	16.2	3.2	−0.40						25.0	
		202	60	4.5	2.3	−0.33						28.2	
	50	194	90	8.1	0.9	−0.11	*−0.30*						
		226	90	17.7	4.3	−0.41							
		176	90	16.3	3.3	−0.54							
		216	90	3.1	1.0	−0.13							

obtained with succinate, table 5 with pyruvate and glucose. The distance from the anterior end of the organ from which the tissue was cut did not measurably alter values. Even in the bundle of Sachs the Q_{O_2} values are the same as in the main organ.

D. *Histological preparations*

Not only the number of electroplaxes per cm. varied in the different sections but their aspect too changed. A number of these preparations were photographed to show the changes of the voltaic pile and some of them are reproduced (see photos 1–7). Since the voltage per electroplax in the small specimen is slightly higher than in the larger fishes, the pictures illustrate why the voltaic pile is so much stronger there. They also show how well the anatomical structure corresponds to the chemical and electrical data.

The number of plates was calculated as the mean value of several serial sections of the same block. The results are summarized in Table 7. Each

figure of the third column is calculated from one serial section. In the first two blocks of specimen No. 2, where two rows are given, the result of a second calculation of the same section is given in the second row. The figures show that two calculations of the same serial section agree satisfactorily, whereas the variations, obtained with different sections of the

Table 5. Respiration of the electric organ at different sections between head and caudal end, with glucose and pyruvate as substrate. The O_2 uptake was observed in the experiments with glucose during 90 min. on specimen No. 3, during 60–80 min. on specimen No. 4, and those with pyruvate during 70 min. At 50(58) cm. bundle of Sachs.

Specimen No.	Distance from ant. end (cm.)	Tissue fresh weight (mg.)	Q_{O_2}	
			single	average
		Glucose		
3	10	198	−0.36	*−0.29*
		186	−0.22	
	30	200	−0.27	*−0.24*
		146	−0.21	
	50	223	−0.54	*−0.34*
		226	−0.26	
		196	−0.41	
		213	−0.15	
4	10	278	−0.58	*−0.42*
		240	−0.31	
		297	−0.39	
		217	−0.41	
	30	223	−0.27	*−0.33*
		284	−0.30	
		236	−0.43	
	58	255	−0.39	*−0.46*
		252	−0.51	
		265	−0.48	
		Pyruvate		
3	10	217		*−0.32*
	30	195		*−0.28*
	50	202		*−0.33*

same block, were more marked. In one case only, in the third block of specimen No. 2, there are two figures, 245 and 246, which are considerably higher than all the others. It may be that these two sections belong to the main organ whereas the other sections belong to the bundle of Sachs.

Discussion

The experiments offer new evidence for the close correlation between

Ch.E. activity and the action potential. V per cm. and QCh.E. run parallel, not only if measured on the same specimen, but even if compared in fishes of different size and with considerable differences of the E. M. F. of the discharge. The parallelism appears therefore to be so close, even in absolute amounts, that the enzyme concentration may be predicted from the voltage and *vice versa* with good approximation.

The voltage per electroplax has been measured only in a few cases. No

Table 6. Number of electroplaxes in the different sections of the electric organs of the four specimens examined.

Specimen No.	D	Number of electroplaxes counted	Average per cm.	
1	3–4	128, 130, 142	133	
	12–13	111, 117, 114, 126, 125	119	
	16.5–17.5	63, 75, 78, 71	72	
2	3.5–5.5	281, 284, 280, 297, 306 286, 296, 289, 299, 302	146	
	11.5–13.5	260, 256, 251, 264, 279 251, 260, 253, 268, 282	131	
	18.5–20.5	182, 181, 185, 216, 217, 245, 246	105	
	25.5–27.5	87, 120, 104 90, 115	48	
3	25.5–26.5	53, 45, 49, 52, 51, 48, 39	48	
	45.5–46.5	5, 5, 5, 7, 8, 10, 9, 10	7	b.o.S.
	55.5–56.5	6, 5, 8, 8, 8, 9, 8	7	b.o.S.
4	5–6	76, 71, 66, 65, 58, 54	65	
	25–26	40, 41, 40, 40	40	
	40–41	26, 24, 18, 16	21	
	55–56	12, 10, 8, 7	9	b.o.S.
	70–71	9, 8, 7, 8, 7	8	b.o.S.

final conclusions are therefore possible. It seems that the voltage per plate is not uniform. It would be desirable to know whether the concentration of enzyme per electroplax changes correspondingly. From the few data available it appears as if there may be some relationship between the concentration of enzyme and voltage per electroplax. However, the parallelism is not as well established as between V per cm. and QCh.E. More data are necessary to elucidate this question.

The concept that the activity of Ch.E. is intrinsically connected with the discharge is emphasized by the fact that this correlation appears to be specific. Respiration which is also necessary for nerve activity, does not vary from the head to the caudal end in sharp contrast to the variations of the QCh.E. values. Other enzymes and substances, examined so far, which will be described in following papers, do not show any parallelism with voltage.

It would be interesting to measure the resting respiration of the intact organ and to compare it with the respiration after activity. It is probable that after activity the oxygen uptake rises and it is possible that it rises somewhat more near the head than at the caudal end. In stimulated nerves the oxygen uptake is about twice as high as in the resting nerve. But the following consideration can be applied. Oxidation is a slow process. In nerve and electric tissue the rate is particularly low. If the QCh.E. is 150–250, 5–10000 times more molecules of ACh can be split per unit of tissue and per unit of time, than molecules of oxygen can be taken up. Even these figures do not indicate the real ratio between the rate of ACh hydrolysis and oxidation. For the QCh.E. values are calculated per unit of weight of tissue whereas it is known that ACh metabolism occurs only at or near the surface of the neurone, that is within a minute fraction of the actual tissue weight. This correction has not to be made for oxidation. Since electrical phenomena occur at a high speed, the high rate of ACh hydrolysis is in itself suggestive, if compared with the low respiration rate and is certainly closer to the discharge than oxidation which may be the last link of a long chain of reactions connected with the action potential.

It will be noticed that it is the voltage per cm. which has been correlated with the QCh.E., whereas the release of ACh may also have to do directly with the alteration of the resistance during the discharge (5). The values of the resistance of the segments of the organ (Table 2) cannot be interpreted without consideration of the cross-sectional area of the segments as well as their lengths. For it is only this specific resistance which might be expected to have some correlation with the chemical reactions of the tissue. The cross sections of the segments have been measured in most of the specimens and the resistance of the organ will be discussed in a following paper. It is at any rate clear that the activity of Ch.E. is closely connected with the electrical activity, both from the results here reported and others to which reference has been made. It is also certain, that a change in the resistance of the tissue is an important feature of the electrical action. Whether the change in resistance is accompanied by a change in E. M. F. and what is the immediate effect of the release of ACh on the electrical quantities are questions on which more experiments are needed.

Summary

1. Voltage, amperage and resistance have been recorded in the electric organ of four specimens of *Electrophorus electricus*, at different sections between head and caudal end. The data were compared with the concentration of Ch.E. at the same segments. V per cm. and QCh.E. run parallel not only in the same fish, but even in fishes of different size in which the E. M. F. of the discharge differs considerably. The experiments offer new evidence for the close relationship between E. M. F. and Ch.E. activity.

2. The respiration has been measured in different sections of the electric organ with different substrates; glucose, pyruvate, and succinate. With

succinate both the succinic oxidizing system and succinic dehydrogenase were examined. In contrast to the great variations of QCh.E. the Q_{O_2} values are practically the same from the head to the caudal end. Even between the strong main organ and the weak bundle of Sachs there is no measurable difference. The oxygen uptake was about the same with all the substrates used.

3. Histological preparations were made from different sections. The number of electroplaxes in series has been calculated. Photographs of the histological preparations, some of which are reproduced in the paper, demonstrate the different aspect of the voltaic pile. They show that not only the number of electroplaxes per cm. varies, but that there are also great varieties of the aspect of the single elctroplax.

We are greatly obliged to Dr. M. Vertner Brown, Department of Physics, College of the City of New York, for help in making the electrical measurements.

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INDEX

Abnormalities, postural, and central excitatory state, 393
Accommodation, breakdown of, 55
Acetylcholine, relation of fibrillation to, in denervated skeletal muscle, 357
ACHESON, G. H., LEE E. S., and MORISON, R. S., A deficiency in the phrenic respiratory discharges parallel to retrograde degeneration, 269
Acidosis, effect on cortical electrical activity and blood flow, 261
Acoustic area of monkey, 49
Activity, cortical, effects of intensity and wave length on, 483
ADES, H. W., and FELDER, R., The acoustic area of the monkey (*Macaca mulatta*), 49
Alkalosis, effect on cortical electrical activity and blood flow, 261
ANDERSON, R., See DOW, R. S., 363
ARVANITAKI, A., Effects evoked in an axon by electric activity of a contiguous one, 89
Audiogenic seizures in rat, 185
Axon, electric activity of, 89

BERNHARD, C. G., Isolation of retinal and optic ganglion response of the eye in *Dytiscus*, 32
——, ——, and GRANIT, R., Stimulus frequency as a means of analyzing synaptic activity, 381
——, ——, ——, ——, and SKOGLUND, C. R., The breakdown of accommodation-nerve as model sense-organ, 55
Blood flow, effects of alkalosis and acidosis on, 261

CAMPBELL, B., See GRUNDFEST, H., 275
Cat, cerebellar action potentials in, 121
——, dorsal spino-cerebellar tracts, 275
Cerebellar action potentials and cerebral cortex, 121
——, in rat, 363
lesions, positional nystagmus, 247
Chimpanzee, section of medial lemniscus, 69
Choline esterase and respiration, 499
COATES, C. W., See NACHMANSOHN, D., 499
CORBIN, K. B., See HARRISON, F., 465
Cortex, cerebral, cerebellar action potentials and, 121
——, ——, effect on electroencephalogram of lesions of, 335
——, ——, excitability of in infant monkey, 231
Cortical activity, modification by stimulation, 349
Cortical electrical activity, effect of alkalosis and acidosis on, 261
COX, R. T., See NACHMANSOHN, D., 499

Degeneration, retrograde, and phrenic respiratory discharges, 269
DENSLOW, J. S., and HASSETT, C. C., The central excitatory state associated with postural abnormalities, 393
Dorsal root, potentials recorded from, 423
DOW, R. S., Cerebellar action potentials in response to stimulation of the cerebral cortex in monkeys and cats, 121
——, ——, and ANDERSON, R., Cerebellar action potentials in response to stimulation of proprioceptors and exteroceptors in the rat, 363
Dytiscus, retinal and optic ganglion response, 32

ECCLES, J. C., KATZ, B., and KUFFLER, S. W., Effect of eserine on neuromuscular transmission, 211
Electric activity of axon, 89
—— changes at nerve muscle junction, 18
Electroencephalogram of monkey, 325, 335
Enzymes, localization of, in nerves, 109
Eserine, effect on neuromuscular transmission, 211
Excitability of cerebral cortex in monkey, 231
Excitation of phrenic motor neurons, 75
Exteroceptors, stimulation of in rat, 363
Eye, retinal and optic ganglion response, 32

FELDER, R. See ADES, H. W., 49
Fibrillation, relation to acetylcholine and potassium, 357
FINGER, F. W., See LINDSLEY, D. B., 185
FOERSTER, OTFRID, *obituary*, 1
Frog, intersegmental inhibition in, 459
Frontal lobes, delayed response in monkey after removal of, 295

Ganglia, basal, effect on electroencephalogram of lesions, 335
GRAHAM, H. T., The effects of polarization on nerve action potentials, 137
GRANIT, R., See BERNHARD, C. G., 55, 381
GRUNDFEST, H., and CAMPBELL, B., Origin, conduction and termination of impulses in dorsal spino-cerebellar tracts of cats, 275

HALSTEAD, W. C., KNOX, G. W., and WALKER, A. E., Modification of cortical activity by means of intermittent photic stimulation in the monkey, 349
——, ——, ——, ——, WOOLF, J., and WALKER, A. E., Effects of intensity

and wave length on driving cortical activity in monkeys, 483
HARRISON, F., and CORBIN, K. B., Oscillographic studies on the spinal tract of the fifth cranial nerve, 465
HASSETT, C. S., See DENSLOW, J. S., 393
HENRY, C. E., See LINDSLEY, D. B., 185

Impulses in dorsal spino-cerebellar tracts in cats, 275
Inhibition, intersegmental, in spinal cord, of frog, 459
—— of phrenic motor neurons, 75
Iris, responses to prolonged stimulation, 27

KATZ, B. Impedance changes in frog's muscle associated with electronic and "endplate" potentials, 169
——, ——, See ECCLES, J. C., 211
KENNARD, M. A., and MCCULLOCH, W. S., Excitability of cerebral cortex in infant *Macaca mulatta*, 231
——, ——, and NIMS, L. F., Changes in normal electroencephalogram of *Macaca mulatta* with growth, 325
——, ——, ——, ——, Effect on electroencephalogram of lesions of cerebral cortex and basal ganglia in *Macaca mulatta*, 335
KNOX, G. W., See HALSTEAD, W. C., 349, 483
KUFFLER, S. W., Electric potential changes at an isolated nerve-muscle junction, 18
——, ——, Further study on transmission in an isolated nerve-muscle fibre preparation, 309
——, ——, Responses during refractory period at myoneural junction in nerve-muscle fibre preparation, 199
——, ——, See ECCLES, J. C., 211

LEE, E. S., See ACHESON, G. H., 269
Lemniscus, medial, effect of section of, 69
LINDSLEY, D. B., FINGER, F. W., and HENRY, C. E., Some physiological aspects of audiogenic seizures in rats, 185
LLOYD, D. P. C., Mediation of descending long spinal reflex activity, 435
——, ——, Stimulation of peripheral nerve terminations by active muscle, 153
LUBIN, A. J., and PRICE, J. C., Effect of alkalosis and acidosis on cortical electrical activity and blood flow, 261
LUCO, J. V., and SALVESTRINI, H., Responses of the iris to prolonged stimulation of its parasympathetic nerve supply, 27

MCCULLOCH, W. S., See KENNARD, M. A., 231
MACHADO, A. L., See NACHMANSOHN, D., 499
MAGLADERY, J. W., and SOLANDT, D. Y., Relation of fibrillation to acetylcholine and potassium sensitivity in denervated skeletal muscle, 357
——, ——, See SOLANDT, D. Y., 373
MALMO, R. B., Interference factors in delayed response in monkeys after removal of frontal lobes, 295
Monkey, acoustic area of, 49
—— cerebellar action potentials in, 121
—— driving cortical activity in, 483
—— electroencephalogram of, 325, 335
—— infant, excitability of cerebral cortex in, 231
—— interference factors in, after removal of frontal lobes, 295
—— intermittent photic stimulation in, 349
—— lesions of cerebral cortex and basal ganglia, 335
—— section of medial lemniscus in, 69
MORISON, R. S., See ACHESON, G. H., 269
Motoneuron, effects of presynaptic volleys, 235
Muscle, impedance changes in frog, 169
——, denervated skeletal, relation of fibrillation, 257
——, skeletal, effect of motor neuron lesions on, 373
——, stimulation of peripheral nerve terminations by, 153

NACHMANSOHN, D., COX, R. W., COATES, C. W. and MACHADO, A. L. Action potential and enzyme activity in the electric organ of *Electrophorus electricus* (Linnaeus). I. Choline esterase and respiration, 499
NACHMANSOHN, D., and STEINBACH, H. B., Localization of enzymes in nerves. I. Succinic dehydrogenase and vitamin B_1, 109
Nerve action potentials, effect of polarization, 137
——, crural, reflex discharges in, 487
——, fifth cranial, oscillographic studies on spinal tract of, 465
——, localization of enzymes in, 109
——, model sense-organ, 55
—— peripheral terminations, 153
—— trunk, potentials recorded from, 423
Nerve-muscle fibre preparation, responses at myoneural junction, 199
——, transmission in, 309
Nerve-muscle junction, electric changes, 18
Neuromuscular transmission, effect of eserine on, 211
Neuron, motor, effect of lesions of, 373
——, phrenic motor, 75

NIMS, L. F., See KENNARD, M. A., 325, 335
Nystagmus, positional in cerebellar lesions, 247

Oscillographic studies of fifth cranial nerve, 465

Pain, visceral responses to, 417
PARRACK, H. O., Potentials recorded from the nerve trunk and the dorsal root by micro-electrodes, 423
Phrenic respiratory discharges, 269
PITTS, R. F., Excitation and inhibition of phrenic motor neurones, 75
——, ——, The function of components of the respiratory complex, 403
Polarization and nerve action potentials, 137
Potassium, relation of fibrillation to, 357
——, and denervated skeletal muscle, 357
Potentials, electronic and "endplate," 169
Presynaptic volleys, effect on spread of impulses of motoneuron, 235
PRICE, J. C., See LUBIN, A. J., 261
Proprioceptive functions, effect of section of medial lemniscus on, 69
Proprioceptors, stimulation of in rat, 363

Rat, audiogenic seizures in, 185
——, cerebellar action potentials in, 363
——, stimulation of exteroceptors in, 363
——, stimulation of proprioceptors in, 363
Reflex activity, spinal, 435
—— discharges of crural nerve, 487
Refractory period, responses during, 199
RENSHAW, B., Effects of presynaptic volleys on spread of impulses over the soma of the motoneuron, 235
——, ——, Reflex discharges in branches of the crural nerve, 487
Respiratory complex, function of components, 403
Response, delayed, in monkey, interference factors, 295

SALVESTRINI, H., See LUCO, J. V., 27
SATTLER, D. G., Absence of local sign in visceral responses to pain, 417
SCALA, N. P., See SPIEGEL, E. A., 247
SJÖQVIST, O., and WEINSTEIN, E. A., The effect of section of the medial lemniscus on proprioceptive functions in chimpanzees and monkeys, 69
SKOGLUND, C. R., See BERNHARD, C. G., 55
SOLANDT, D. Y., and MAGLADERY, J. W., A comparison of effects of upper and lower motor neurone lesions on skeletal muscle, 373
——, ——, See MAGLADERY, J. W., 357
SPIEGEL, E. A., and SCALA, N. P., Positional nystagmus in cerebellar lesions, 247
Spino-cerebellar tracts, dorsal, 275
STEINBACH, H. B., See NACHMANSOHN, D., 109
Stimulus frequency and synaptic activity, 381
Stimulation, intermittent photic, in monkey, 349
Succinic dehydrogenase, 109
Synaptic activity and stimulus frequency, 381

TERZIOGLU, M., See WINTERSTEIN, H., 459

Visceral responses to pain, 417
Vitamin B_1, 109

WALKER, A. E., See HALSTEAD, W. C., 349, 483
WEINSTEIN, E. A., See SJÖQVIST, O., 69
WINTERSTEIN, H., and TERZIOGLU, M. Intersegmental inhibition in the spinal cord of frog, 459
WOOLF, J., See HALSTEAD, W. C., 483

To THOSE readers of the Journal who are interested in poliomyelitis, Dr. H. C. Elliott of the Department of Anatomy, University of Toronto, Toronto, Canada, wishes to make an appeal.

He is following what seems a promising line of research on this disease and finds himself greatly handicapped by lack of material. Spinal cords from cases of the disease, acute and even more particularly chronic, are needed. A single cord would be of major value, and he appeals particularly to individual physicians and surgeons, and to small hospitals.

Requirements are: Intact cords, or intact upper or lower halves; dura left on but slit up the back; cord may be cut into equal thirds. Fixation preferably with 2% glacial acetic in 95% alcohol, but may be with 10% formalin in normal saline; use about a pint; please fix straight. Up to ten dollars can be supplied to pay technical assistance in removing a cord, and all expenses will be paid. If sections are required for local records Doctor Elliott will be glad to prepare these himself—if donor removes blocks for this purpose it impairs the value of the material. Correspondence is cordially invited. Material will be required till Summer of 1943 or later.

TABLE OF CONTENTS

VOL. V, No. 6

JOURNAL OF NEUROPHYSIOLOGY

1. DWIGHT G. SATTLER. Absence of local sign in visceral responses to pain..... 417

2. HORACE O. PARRACK. Potentials recorded from the nerve trunk and the dorsal root by micro-electrodes..... 423

3. DAVID P. C. LLOYD. Mediation of descending long spinal reflex activity..... 435

4. HANS WINTERSTEIN AND MELIHA TERZIOGLU. Intersegmental inhibition in the spinal cord of the frog..... 459

5. FRANK HARRISON AND KENDALL B. CORBIN. Oscillographic studies on the spinal tract of the fifth cranial nerve..... 465

6. WARD C. HALSTEAD, GEORGE W. KNOX, JACK I. WOOLF AND A. EARL WALKER. Effects of intensity and wave length on driving cortical activity in monkeys..... 483

7. BIRDSEY RENSHAW. Reflex discharges in branches of the crural nerve..... 487

8. DAVID NACHMANSOHN, RICHARD T. COX, C. W. COATES, AND A. L. MACHADO. Action potential and enzyme activity in the electric organ of *Electrophorus electricus* (Linnaeus). I. Choline esterase and respiration..... 499